

Volume 35

**Advances in
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Advances in Pediatrics®
Volume 35

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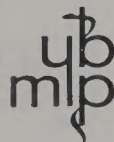
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Preface

We must be picking the right authors or the right subjects. We have never received so many comments, mostly laudatory, for *Advances in Pediatrics* as in the past 3 years. When this was mentioned to the publishers 3 years ago, they thought it might be a fluke. But the continued increase in comments from practitioners, residents, investigators, and a few medical students is highly gratifying. There has been a similar increase in requests for permission to use material from previous volumes for other publications; this volume promises to be equally as useful and informative.

Every once in a while, one reads an article that explains all sorts of phenomena one would like to understand. Stiehm has written such an excellent exposé on the gammaglobulins. Not only does he summarize the new uses of the gammaglobulins, but he explains why they work. This is must reading.

Rosenbaum and Leibel have put together, not only from their own remarkable studies but also from the work of others, a magnificent review of scientific understanding of obesity. This is required study for all those engaged in the care of obese children. At the least, it provides understanding for the high rate of recidivism.

An exciting chapter in understanding pulmonary and other diseases brings together clinical data of long-ago-described syndromes and new techniques. Carson and Collier carefully take us through the effect of cilia abnormalities in multiple diseases and hopefully provide the first giant step in preventing or curing them.

Only 4 years ago (*Advances in Pediatrics*, Volume 32) Winter and MacLaren summarized immunotherapy in insulin dependent diabetic children. Now, Riley, MacLaren, and Silverstein offer the basis for predicting response to immunotherapy and give further evidence of the immunological basis of diabetes.

Lemanske and Sampson contribute an exciting understanding of food allergy as related to skin manifestations. Their thorough review provides insight into methods of testing, immunologic bases, places for immunotherapy, food additives, significance of breast feeding as well as proper treatment of urticaria, atopic eczema, and dermatitis herpetiformis.

This paper is followed by an update of pathogenesis and treatment of gastrointestinal syndromes associated with food sensitivity by Proujansky, Winter, and Walker. This follows the article from the same group 7 years ago (*Advances in Pediatrics*, Volume 28).

Pediatricians have been bombarded the past few years with questions about indoor air pollutants. Angle has made a masterful collation of an-

swers to these questions, as well as provided some fundamental data on causation, almost suggesting that it's best not to breathe. Six years ago, she with McIntire recommended care in what we eat when discussing lead poisoning (*Advances in Pediatrics*, Volume 29).

Are we creating Alzheimer's disease by drinking from aluminum cans? Gruskin doesn't answer this question, but he gives a reasoned report on the evidence for aluminum toxicity, the many diseases it causes, how to avoid them (especially those that are doctor-induced) and treatment of such diseases.

In one of the most thorough reviews of the diagnosis of iron deficiency and its diagnosis, Reeves et al. (*Advances in Pediatrics*, Volume 30) carefully delineated the care needed and difficulty in making a diagnosis of iron deficiency. With the multiple syndromes attributed to iron deficiency, Lozoff sorts out the significance of various claims and comes to some surprising conclusions.

This is followed by a chapter on one of the important red cell producers. The availability of a genetically engineered erythropoietin allows better understanding of its effects and functions. Chandra, McVicar, and Clemons present the metabolic and physiologic significance of this substance in chronic renal failure and its expected uses. These investigators recently wrote on pathogenic mechanism in the nephrotic syndrome (*Advances in Pediatrics*, Volume 32).

Treatment of osteogenesis imperfecta, while far from perfect, has improved. Even more striking, however, is the study as detailed by Marini, in a delightfully understandable fashion, of the importance of the genetics of this disorder in opening new vistas of inheritance patterns.

Methods of measuring blood flow have recently been inundated with technology to the benefit of understanding physiology. Berman, Lister, Pitt, and Hoffman have combined to write a cohesive description of the new techniques to clarify applications of these methods.

Persistent diarrhea in young children of normal weight, also termed "chronic diarrhea of infancy," "irritable colon," or "toddlers diarrhea," is a common practical problem. Andres redefines the problem and its significance and in addition discusses its differential diagnosis with new material obtained from observations in day-care centers. (Rossi T, Lebenthal E, *Advances in Pediatrics*, Volume 30).

In a departure from the emphasis on advances, the editors have selected a review paper with only a few advances. We felt this was necessary because the safety and availability of oral rehydration (Levin M, Pizzaro D, *Advances in Pediatrics*, Volume 31) has made the careful calculations and monitoring of intravenous therapy less of a necessity. Feld, Kaskel, and Schoeneman have nicely brought up-to-date basis of requirements for parenteral fluid therapy, having previously written a superb review on renal replacement therapy (*Advances in Pediatrics*, Volume 32).

The 14 articles here contain so many new ideas and new approaches that summary does not do justice. However, in addition to the advances,

the reading hopefully is enjoyable and the text will remain a significant reference source.

In addition to welcoming the new Assistant Editor, Dr. Darryl C. DeVivo, an outstanding pediatric neurologist, we thank Dr. John I. Malone for his review of several articles and his pithy comments.

Lewis A. Barness, M.D.

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Human Gamma Globulins as Therapeutic Agents

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Passive immunity (administration of antibodies rather than antigens) for the prevention of infectious disease dates from the turn of the century, e.g., the injection of equine serum for prevention of diphtheria¹ and canine serum for prevention of rabies (Table 1).² Human "convalescent" serum was first used in the early 1900s for the prevention of measles,³ pertussis,⁴ and mumps.⁵ Use of the antibody-rich γ -globulin fraction of human serum dates from World War II.⁶ Alcohol "Cohn" fractionation of pooled human serum concentrated the antibodies, eliminated viruses, and provided a sterile stabilized product with predictable biologic activity. This product, standard human immune serum globulin (HISG), was used shortly thereafter for the prevention of hepatitis A, measles, and poliomyelitis.⁶ Special HISGs, derived from immunized or convalescing donors with high titers of antibody, were first prepared for mumps, vaccinia, and pertussis and subsequently for several other diseases (Table 2). Following the discovery of agammaglobulinemia in 1952,⁷ large quantities of standard HISG were used for replacement therapy in this and other antibody immunodeficiencies.

Thus, standard and special HISGs have held an important therapeutic niche in the prevention and treatment of certain disorders for many years. They are among the safest biologics, with very few adverse side reactions. Their chief disadvantage is the necessity to give them intramuscularly or subcutaneously; their intravenous use is associated with severe anaphylactoid reactions, mainly a result of in vivo complement activation. This limits the quantity of material that can be given; indeed patients with agammaglobulinemia need at least 0.7 ml/kg/month of standard HISG, or 50 ml for a 70-kg adult, an enormous and painful quantity of fluid to be injecting repeatedly.

Accordingly, several pharmaceutical companies sought to develop human γ -globulin preparations that could safely be given intravenously; this was first achieved in 1981 with the licensure by Cutter Laboratories of Gamimune.⁸ Since then several other companies in the United States and abroad have developed similar products for intravenous use.

TABLE 1.
Preparations Available for Passive Immunity*

Animal antiserum and antitoxins
 (for example, diphtheria antitoxin)
Human immune serum globulins for general use
 Immune globulins for intramuscular use
 Immune globulins for intravenous use
Special human immune serum globulins
 (for example, hepatitis B immune globulin)

*From Stiehm ER: Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367. Used by permission.

TABLE 2.
Special Human Immune Serum Globulins Available

Product	Abbreviation	Use
Hepatitis B immune globulin	HBIG	Prevention of hepatitis B
Varicella-zoster immune globulin	VZIG	Modification or prevention of chickenpox
Rabies immune globulin	RIG	Prevention of rabies
Tetanus immune globulin	TIG	Prevention or treatment of tetanus
Vaccinia immune globulin	VIG	Prevention or treatment of smallpox, vaccinia
Pertussis immune globulin		Treatment of pertussis, efficacy doubtful
Rho(D) immune globulin	Rho-GAM	Prevention of Rh hemolytic disease of newborn

This product, immune globulin, intravenous (IGIV), has proven to be an excellent agent in the therapy of antibody deficiency, idiopathic thrombocytopenic purpura, Kawasaki's disease, and several other immunologic and infectious disorders.

In this review, the present uses of standard, special, and intravenous human immunoglobulins will be outlined and some future uses for such products will be described.

Properties of Human IgG Globulin

The immunoglobulins or γ -globulins are the proteins of the plasma and tissue made in lymphoreticular tissues that have antibody activity.⁹ Although there are six classes of immunoglobulin—IgG, IgM, IgA, IgD, IgE, and secretory IgA—only IgG is present in significant quantities in HISSG.

IgG is a glycoprotein with a molecular weight of 150,000 daltons distributed equally between the serum and the tissues. The IgG molecule is Y-shaped with two combining sites, one at the end of each arm. It is made up of two heavy (γ) chains and two light (κ or λ) chains held together by disulfide bonds and weak covalent forces. Its structure is illustrated in Figure 1.

There are four subclasses of IgG—IgG1, IgG2, IgG3, and IgG4—composing about 66%, 23%, 7%, and 4%, respectively, of the total serum IgG (Table 3). Most normal persons have IgG molecules of all subclasses. These subclasses have minor antigenic differences in the heavy chains, resulting in important biologic differences. For example, the IgG3 subclass has a shorter half-life (7 days vs. 21 days), IgG2 is more resistant to pro-

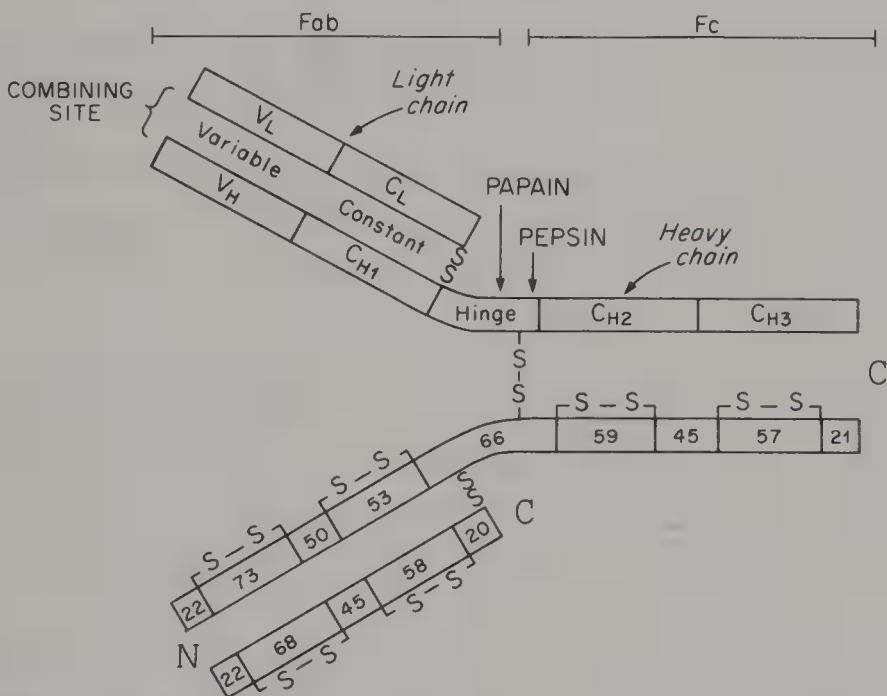


FIG 1.

An IgG molecule. N indicates aminoterminal end and C indicates the carboxyterminal end. Intrachain and interchain disulfide bonds are indicated by arrows. Each chain consists of one variable and several constant segments. Numbers on lower chains refer to the number of amino acid residues composing each portion of the molecule.

TABLE 3.
Properties of Human IgG Subclasses

	IgG1	IgG2	IgG3	IgG4
Relative content in serum (%)	66	23	7	4
Serum half life (days)	21	21	77	21
Deficiency levels (mg/dl)	<300	<100	<40	0
Transplacental transfer	+	+	+	+
Complement binding	+++	+	+++	0
Monocyte binding	+	0	+	0
Staph A binding	+	+	0	+
Mast cell binding	0	0	0	+
Gm factors	Many	Gm 23 only	Many	0
Antibody to protein antigens	+++	++	+	++
Antibody to polysaccharide antigens	+	+++	0	+

teolytic digestion, each genetic IgG (Gm) factor resides in only one of the subclasses, IgG4 cannot bind to complement, IgG2 has most of the antibodies to polysaccharide antigens, etc. The distribution of the IgG subclasses in HISC approximates that of serum. Deficiencies of IgG subclasses, despite near normal total IgG, have been described.

In addition to subclass heterogeneity, there is genetic heterogeneity of IgG molecules, the Gm types, Gm1, Gm2, Gm20, etc. These are due to minor antigenic differences (often due to a single amino acid difference) of the heavy chains under genetic control. Unlike the IgG subclasses, an individual will have a limited number of the 25 known Gm types, based on Mendelian genetics. An individual exposed to an IgG of a Gm type that he or she lacks may develop an antibody (an anti-Gm) to the foreign Gm.

IgG has a mean half-life of 20 days, the longest half-life of any plasma protein (the mean being the average of all of the IgG subclasses). The synthetic rate of IgG is 35 mg/kg of body weight per day, equivalent to 2 gm of IgG synthesized by a 70-kg adult. IgG metabolism is regulated by the serum level of IgG, with an increase in catabolic rate with high levels of IgG and a decrease at low levels. Thus, the IgG half-life of patients with agammaglobulinemia is prolonged to 35 to 40 days.

IgG is made primarily in plasma cells that have differentiated from circulating B-lymphocytes. These are located in central lymphoid tissues (lymph nodes, bone marrow, spleen, and liver), and peripheral lymphoid tissues (secretory glands, intestinal wall). IgG destruction occurs within granulocytes (after phagocytosis of IgG-coated bacteria and particles) in the reticuloendothelial system, and in the gastrointestinal tract. IgG readily

crosses the placenta during late pregnancy, providing passive immunity to the newborn for about 6 months.

IgG, comprising about 80% of the serum immunoglobulin (mean adult serum level, 1,200 mg/dl), is the chief component of the body's serologic defenses. It contains most of the antibacterial, antiviral, antiprotozoal, and antitoxic activity of the serum. Many cells (e.g., lymphocytes, macrophages, killer cells, some granulocytes) have a receptor for the nonantibody (Fc) portion of IgG, permitting attachment of IgG to these cells. The presence of antibody on target cells (tumor cells, heterologous erythrocytes, allogeneic lymphocytes) may permit antibody-dependent cytotoxicity by some lymphocytes, neutrophils, and macrophages.

IgG can also activate the complement system, promote opsonization, and participate in antibody-dependent cytolytic reactions. The distribution of IgG within the tissues permits its participation in extravascular immune reactions.

IgG antibodies are potent inhibitors and competitors of other immune responses. Newborns do not form antibodies to vaccine antigens (e.g., measles) if they have received significant maternal antibody to that antigen. The inhibition of Rh sensitization by passive administration of Rh antibodies is used to prevent Rh hemolytic disease of the newborn. Successful allergy desensitization partially results when highly avid IgG-blocking antibodies develop; these prevent an allergen from reacting with an IgE-coated mast cell and initiating an allergic response.

Use of Standard Human Immune Serum Globulin

Standard HISG for intramuscular administration is used for the treatment of antibody deficiencies (Table 4) and for the prevention or modification of several infectious disorders (Table 5).

Pharmacology of HISG

HISG is prepared by alcohol fractionation of pooled human serum by Cohn's alcohol fractionation procedure (thus deriving its alternative name of Cohn fraction II). This procedure removes most other serum proteins, hepatitis viruses, and human immunodeficiency virus (HIV), thus providing a safe product for intramuscular injection. It is reconstituted as a sterile 16.5% solution (165 mg/ml) with thimerosal as a preservative.* It contains a wide spectrum of antibodies to viral and bacterial antigens.

HISG is 95% IgG globulin, but trace quantities of IgM and IgA and other serum proteins are present. The IgM and IgA are therapeutically insignificant because of their short half-lives (about 7 days) and their low concentrations. HISG contains all IgG allotypes (Gm and Km types).

HISG is approved only for intramuscular or subcutaneous use; intrave-

*In the United States. In many other countries, HISG is preservative free.

TABLE 4.
Immunodeficiencies in Which Immunoglobulin May
Be Beneficial*

Antibody deficiencies
X-linked agammaglobulinemia
Common variable immunodeficiency
Transient hypogammaglobulinemia of infancy (sometimes)
IgG subclass deficiency +/- IgA deficiency (sometimes)
Antibody deficiency with normal immunoglobulin levels
Combined deficiencies
Severe combined immunodeficiencies (all types)
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
Short-limbed dwarfism
X-linked lymphoproliferative syndrome
Secondary immunodeficiencies
Protein-losing enteropathies with hypogammaglobulinemia
Malignancies with antibody deficiencies; multiple myeloma, chronic lymphocytic leukemia
Nephrotic syndrome
Pediatric acquired immunodeficiency syndrome
Burns
Prematurity

*From Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367. Used by permission.

nous injections of HISG are contraindicated. It aggregates in vitro to large-molecular-weight complexes (9.55 to 40 S), which are strongly anticomplementary. These aggregates are probably responsible for the occasional systemic reactions to HISG. The incidence of these reactions is increased if the recipient has received γ -globulin previously or if given intravenously. Agammaglobulinemic boys with affected male relatives (suggesting X-linked inheritance) may have a lower incidence of reactions.¹⁰ Small intradermal injections of HISG are not of value (except as a placebo), and they are also contraindicated.

The maximum increase of the serum IgG level after a standard HISG injection will vary from patient to patient and from dose to dose because of different rates of absorption, local proteolysis at the injection site, and distribution within the tissues. An intramuscular injection of 100 mg/kg of HISG usually raises the IgG serum level by 100 mg/dl after 2 to 4 days.¹¹ Thus, a recent HISG injection usually does not obscure the diagnosis of agammaglobulinemia or hypogammaglobulinemia.

TABLE 5.
Indications for and Dosage of Standard Human Immune Serum Globulin for Intramuscular Use*†

Illness	Goal	Dose	Comments
Antibody immunodeficiencies	Treatment	0.7 ml/kg every 2–4 weeks	Double dose at onset of therapy, give at multiple sites
Hepatitis A	Prevention—single exposure	0.02–0.04 ml/kg	Higher dose in adults, heavy exposure
	Prevention—continuous exposure	0.02–0.06 ml/kg	Repeat in 4–5 months if exposure continues
Hepatitis B	Prevention	0.06–0.12 ml/kg	Use if HBIG is unavailable or exposure uncertain
Hepatitis non-A, non-B	Prevention	0.12 ml/kg	Use with transfusions under special circumstances
Measles	Prevention	0.25–0.50 ml/kg	Higher dose in immunocompromised patient
Chickenpox	Modification	0.05 ml/kg	Use in high-risk patients if VZIG is unavailable or unaffordable
	Modification	0.6–1.2 ml/kg	Use only during pregnancy with rubella titers; efficacy unreliable
Rubella	Prevention	0.55 ml/kg	Rarely indicated
Poliomyelitis	Prevention	0.15 ml/kg	

*From Stiehm ER: Passive immunization, in Feigen RC, Cherry JD: *Textbook of Pediatric Infectious Diseases*, ed 2. Philadelphia, WB Saunders Co, 1987, pp 2295–2319. Used by permission.

†HISG is a 16.5% solution (165 mg/ml), for intramuscular use only, available in 2- or 10-ml vials from several manufacturers.

Adverse Effects of HISG

Although HISG is one of the safest biologic products available, rare anaphylactic reactions to intramuscular injections have been reported, particularly in patients requiring repeat injections.¹² The United Kingdom's Medical Research Council Working Party¹⁰ noted such reactions in 33 of 175 patients (19%) treated over a 10-year period. In all there were 85 reactions to about 40,000 injections; in eight patients the injections were stopped as a result of these adverse effects, and one death was recorded. Such reactions occurred at any stage of treatment and were unrelated to any particular lot number of HISG or its anticomplementary activity. The symptoms include anxiety, nausea, vomiting, malaise, flushing, facial swelling, wheezing, cyanosis, and loss of consciousness. Immediate treatment with epinephrine and antihistamines is indicated.

Individuals who experience such reactions should be evaluated prior to a repeat injection. Skin testing using several lots of HISG should be done.¹² A skin test that is positive for an old but not a new lot of HISG may indicate a particular idiosyncratic reaction to a particular unit. Under these circumstances incremental doses of HISG from a new lot are recommended. Other patients develop IgE antibodies to IgG resulting in positive skin tests.¹³ In others, no cause of the reactions can be found. Some of these patients will tolerate repeated small doses of HISG, particularly if they are premedicated with aspirin, diphenhydramine, or corticosteroids. A few patients have developed IgG or IgE antibodies to the IgA present in minute quantities in the HISG. These IgA antibodies can be detected by serologic methods. IGIV with low IgA content or IgA deficient plasma can be used under these circumstances.¹³

Some patients given γ -globulin (or plasma) may develop antibodies to a genetic γ -globulin allotype different from their own (usually anti-Gm antibodies). Allen and Kunkel¹⁴ found anti-Gm antibodies in 17 of 24 thalassemic children given repeated blood transfusions. Stiehm and Fudenberg¹⁵ noted anti-Gm antibodies in normal children given single γ -globulin injections and hypogammaglobulinemic children given repeated γ -globulin injections. Patients with severe antibody immunodeficiency do not develop such antibodies. One plasma transfusion reaction was attributed to a Gm-anti-Gm interaction.¹⁶ Patients with antibodies to IgA may have a reaction to γ -globulin as a result of the tract quantities of IgA in HISG.¹⁷ However some patients with combined IgA and IgG2 deficiency with anti-IgA antibodies tolerate treatment with IGIV, particularly IGIV with low levels of IgA.¹⁸

Administration of exogenous γ -globulin may theoretically inhibit the endogenous synthesis of γ -globulin. In immunodeficiency with hyper-IgM, intramuscular IgG results in diminution of IgM levels, suggesting feedback inhibition of endogenous IgM synthesis.¹⁹ We have noted depressed IgG levels in a few patients with transient hypogammaglobulinemia given HISG from early infancy; these normalize when the injections are stopped.

Late side effects to HISG injections are uncommon; however, some patients develop fibrosis of the buttocks or localized subcutaneous atrophy at the site of repeated injections. Repeated injections of HISG may result in high levels of mercury as a result of the thimerosal preservative. Although one patient developed acrodynia as a result of such therapy,²⁰ most remain asymptomatic.

HISG in Antibody Deficiencies

Patients with primary antibody deficiency, primary combined (T cell and antibody) deficiency, and secondary antibody deficiency may benefit from repeated injections of intramuscular γ -globulin. A list of these disorders was provided in Table 4. In general, all patients with IgG serum concentrations less than 200 mg/dl will need and will benefit from IgG injections. In addition most patients with IgG concentrations between 200 and 400 mg/dl will benefit from IgG injections, although if antibody levels are functionally normal, IgG injections may not be necessary. Any patient with functional antibody deficiency despite the concentration of IgG is also a candidate for γ -G globulin therapy. If platelet counts are less than 20,000/ μ l or a bleeding tendency exists, HISG and other intramuscular injections are contraindicated because of the risk of intramuscular hemorrhage.

The initial decision as to whether IGIV or HISG will be used in the treatment (or continuation of treatment) of antibody deficiency is based on the considerations in Table 13. At similar doses, the agents are therapeutically comparable. Most young infants under age 2, because of poor venous access, need to receive HISG. Many older patients prefer HISG because they have been receiving injections for a long time without difficulty or do not like the long duration for administration or the expense of IGIV therapy.

Dosage

The usual dosage of HISG for antibody immunodeficiency is 100 mg/kg/month, about equivalent to 0.7 ml/kg/month of the commercially available 16.5% (165 mg/ml) product. A double or triple dose is given at the onset of therapy, often over a 3- to 5-day period. The maximum dosage should not exceed 20 or 30 ml/week. Few studies of the optimal dosage are available; however, the Medical Research Council Working Party¹⁰ found that 25 mg/kg/week (100 mg/kg/month) was equivalent therapeutically to 50 mg/kg/week, but that 10 mg/kg/week was inadequate.

The HISG should be given at multiple sites to avoid giving more than 5 ml at any one site (10 ml in a large adult). The buttocks are the preferred sites, but the anterior thighs can also be used. Tenderness, sterile abscesses, fibrosis, and sciatic nerve injury may result from these injections. The danger of sciatic nerve injury is especially great in a small malnourished infant with inadequate muscle and fat in the gluteal regions.

The injections are initially given at monthly intervals. If the patient con-

tinues to have infection or if a characteristic symptom recurs at the end of the injection period (such as cough, conjunctivitis, diarrhea, arthralgia, or purulent nasal discharge), the interval between doses is decreased to 3 or 2 weeks. Older patients often report that they can tell when their IgG level is low and when they need another injection. During acute infections, IgG catabolism increases, so extra injections of HISG should be given.

Since no specific serum level of IgG must be or can be maintained, serial serum IgG assays are generally unnecessary in assessing the effectiveness of HISG treatment.

Slow Subcutaneous HISG Infusions

As an alternative to intramuscular injections for antibody deficiency, HISG can be given to immunodeficient patients by slow (1 to 2 ml/hour) subcutaneous injections.²¹ These injections are self-administered into the abdominal wall with a battery-operated pump (Auto syringe). These injections are well tolerated and enable the patients to get increased quantities of HISG and maintain higher serum levels of IgG. We have utilized this method successfully in a man who had repeated anaphylactic reactions to intramuscular HISG.²²

HISG in Measles

The first successful prophylaxis of measles with convalescent serum was reported by Cenci³ in 1907. Serum was first used in the United States in 1916 by Park, Freeman, and Zingher, who gave 41 recently exposed children at New York Metropolitan Hospital either 4 or 8 ml of convalescent serum.^{23, 24} None of the 20 children receiving the 8-ml dose and three of the 20 children receiving the 4-ml dose developed measles. Park and Freeman²³ in 1926 found that 6 to 10 ml of convalescent serum was 92% efficacious in preventing measles in recently exposed individuals, a finding confirmed and extended by Stillerman et al.²⁵ Placental extracts containing serum antibodies also were used in the prevention and modification of measles.^{26, 27}

Studies by Stokes et al.²⁷ and Ordman et al.²⁸ in 1944 established that (1) large doses (0.05 ml/kg) of HISG given immediately after exposure could prevent measles, (2) lesser doses (0.01 ml/kg) given immediately after exposure could modify measles, and (3) large doses (0.05 ml/kg) given in the early stages of clinical illness could lessen the severity of measles.

Black and Yannet³⁰ in 1960 observed that ten of 38 children given HISG (0.1 ml/lb) during a measles epidemic at a mental institution developed antibodies but had no clinical evidence of disease, suggesting passive-active immunity. Greenberg et al.³¹ in 1955 noted that there was a lower incidence of measles encephalitis in HISG-modified measles.

The next use of HISG in measles was in diminishing side effects of the Edmonston strain of attenuated measles vaccine. Krugman et al.³² in 1962

noted that the simultaneous administration of 0.02 ml/lb of HISG and Edmonston measles vaccine, compared with the vaccine alone, reduced the incidence of high fever from 40% to 14% and the incidence of rash from 10% to 2%. The mean titer of measles antibody achieved was somewhat reduced by the HISG, and there was a slight decrease in the rate of seroconversion; nevertheless, the vaccine-HISG combination was 95% effective.³³ The use of further attenuated measles vaccine has eliminated the necessity for concomitant HISG injections. Further, the widespread use of measles vaccine has greatly reduced the need for HISG for measles prophylaxis.

Recommendations

In nonvaccinated normal infants and children exposed to measles, a preventive dose of HISG (0.25 ml/kg intramuscularly) should be given as soon as possible after exposure. Twelve or more weeks later, measles vaccine should be given for permanent immunity. Alternatively, an attenuating dose of HISG can be given at a lower dose (0.05 ml/kg). In normal infants, 6 to 15 months of age with a high risk of exposure to measles (e.g., an infant traveling to a country where there is endemic measles), 0.25 ml/kg of HISG can be given at 3-month intervals to prevent measles. Vaccination before 15 months of age may not be effective and can lead to a partial state of tolerance to subsequent vaccine.³⁴

High-risk children exposed to measles (those with leukemia, lymphoma, generalized malignancy, immunodeficiency, or taking immunosuppressive drugs) should be given a larger dose (0.50 ml/kg, maximum 15 ml). Immunodeficient children receiving regular doses of HISG do not require additional HISG upon measles exposure.

HISG in Hepatitis A (Infectious Hepatitis)

The widest use of HISG by the pediatric practitioner is in the prevention of hepatitis A. Its efficacy has been demonstrated repeatedly since the 1945 studies of Stokes and Neefe³⁵ aborting an epidemic in a children's summer camp, of Havens and Paul³⁶ controlling an institutional epidemic, and of Gellis and associates³⁷ preventing hepatitis A in the Mediterranean theater of operations at the close of World War II. There is no other effective means of prophylaxis for close family contacts (although scrupulous cleanliness may interrupt the intestinal-oral circuit of transmission when contacts are not using the same hygienic facilities).

HISG is efficacious in hepatitis A if given any time during the incubation period up until 6 days before the onset of disease. The protection persists for a period of 6 to 8 weeks. Stokes et al.³⁸ noted that a single small dose of HISG (0.02 ml/kg) provided a degree of protection for up to 9 months for individuals residing at an institution in which hepatitis A was endemic.

The effectiveness of HISG in hepatitis A varies from 80% to 95%, depending on how soon it is administered after exposure and the severity of

the exposure.³⁹ HISG suppresses the clinical manifestations of the disease, but anicteric hepatitis is not prevented, and the ratio of anicteric hepatitis to icteric hepatitis may be as high as 12:1.⁴⁰ Since the period of protection may exceed the expected duration of the HISG, the concept of passive-active immunity has emerged in which, as a result of continuous exposure, a mild illness ensues and, in turn, confers long-lasting immunity.^{38, 40} This has led to the recommendation that individuals living in an endemic area receive only one additional HISG injection after the initial injection. However, Woodson and Clinton⁴¹ noted several cases of hepatitis A in Peace Corps volunteers serving 2 years in endemic areas, occurring up to 26 weeks after the last HISG injection. They recommended that HISG be given at 4-month intervals for the duration of exposure.

The initial studies of Stokes and Neefe³⁵ employed a dose of 0.15 ml/lb. Other early workers used doses of 0.06 ml/lb to 0.12 ml/lb.^{36, 37} Stokes et al.³⁸ in 1951 showed that doses as little as 0.01 ml/lb were effective in limiting spread but not totally preventing hepatitis. Hsia et al.⁴² in 1954 also noted that a dose of 0.01 ml/lb was effective in preventing hepatitis among family contacts. Ward and Krugman³⁹ in 1958 were able to reduce the incidence of hepatitis in institutional patients from 19.5 cases per 1,000 to 7.4 cases per 1,000 with 0.01 ml/kg and to 1.7 cases per 1,000 with 0.06 ml/kg. The larger dose may be particularly important in adults, since they get more severe disease.

The development of antibody tests for hepatitis A provides a way to determine (1) the immunity of a subject, (2) presence of inapparent infection, (3) the titer of hepatitis A virus in lots of HISG, and (4) the validity of the passive-active immunity concept.^{43, 44} In later studies, Krugman⁴⁵ showed that an HISG preparation with a titer of 1:3,200 by an immune adherence test was effective in neutralizing the infectivity of MS-1 serum, a serum known to contain hepatitis A virus. Among seronegative children who received the HISG-hepatitis A mixture, six remained seronegative and two became seropositive; one became ill. By contrast, eight of 14 children who received MS-1 serum without HISG developed hepatitis.

Most current lots of HISG have antibodies to hepatitis A virus (anti-HAV), determined by a competitive-inhibition radioimmunoassay (RIA). Titers greater than 1:100 are protective. Recent lots of HISG may contain more antibodies to hepatitis A virus than lots obtained 10 years ago.⁴⁶

Recommendations

Individual Exposure.—Individuals, either adults or children, with known intimate exposure to hepatitis A such as a household or child care contact should be given a single dose of 0.02 ml/kg of HISG. This includes newborns of mothers with hepatitis. Serologic testing for hepatitis A positivity is unnecessary and may delay administration of HISG. The use of HISG after 2 weeks of exposure is not indicated.

HISG is usually unnecessary for children exposed to hepatitis A at day school. However, HISG prophylaxis is recommended for children exposed

at a boarding school or in a school for retarded children, where the opportunities for fecal-oral route transmission are increased. Hospitalized children exposed to another child with hepatitis A on the hospital ward need not be given HISG.

Institutional Outbreaks.—Institutional hepatitis A outbreaks such as in boarding schools, day care centers, facilities for the mentally retarded, or prisons require aggressive action. Other cohorts, employees, and adult members of the households of infants who wear diapers and who attend these facilities should be treated immediately with 0.02 mg/kg of HISG. If an outbreak of hepatitis A is traced to a food handler, HISG should be given to his or her close contacts and other restaurant employees.⁴⁷

Foreign Travel.—Ordinary tourist travel does not require HISG prophylaxis. However, individuals going to developing countries should receive 0.02 ml/kg of HISG if they intend to stay less than 3 months. If longer stays are anticipated, testing for immunity to hepatitis A virus is advisable. Nonimmune individuals should receive 0.06 ml/kg of HISG, repeated every 4 to 5 months. Some recommend stopping after one or two supplemental injections, with the hope that active immunization has ensued. It seems relatively innocuous to continue the HISG until there is clinical or serologic evidence that active immunity has been established. Most individuals who reside permanently (longer than 2 years) in endemic areas do not maintain their HISG injections.

Primate Exposure.—Certain subhuman primates such as chimpanzees may carry hepatitis A virus. Animal handlers should observe scrupulous hygiene and be tested for hepatitis A virus antibody. If they are found to be susceptible, prophylaxis with HISG, 0.06 ml/kg every 5 months, is advisable.

Needle Exposure.—HISG is indicated for individuals accidentally inoculated with blood or serum from a patient with hepatitis A. The recommended dose is 0.02 ml/kg of HISG. Pregnancy is not a contraindication to HISG.

HISG in Hepatitis B (Serum Hepatitis)

In contrast to its proven efficacy in the prophylaxis of hepatitis A, HISG is not consistently able to prevent hepatitis B ("serum hepatitis"), thus making a definite recommendation for its routine use impossible. Part of this inconsistency derives from the fact, not appreciated in early studies, that most cases (up to 80%) of posttransfusion hepatitis are not due to the hepatitis B virus.

The initial study of HISG in hepatitis B was conducted in 1945 by Grossman et al.⁴⁸ who treated alternate battle casualties given whole blood or plasma with two 10-ml injections of HISG 1 month apart. The incidence of icteric hepatitis was 1.3% in 384 HISG-treated patients and 9.9% in 384 control patients, a highly significant difference that suggested a beneficial effect of HISG. In 1947, Duncan et al.⁴⁹ reported the results from a

similar study, though they gave only one 10-ml injection; hepatitis occurred in 1.2% of 2,406 patients in the HISG-treated group and 0.9% of patients in the control group, an insignificant difference. Significant prolongation of the mean incubation period in the HISG-treated group (to 103 days) compared with that in the control group (87 days) was noted. Drake et al.⁵⁰ could not demonstrate a beneficial effect of HISG derived from convalescent hepatitis patients when given to volunteers deliberately inoculated with blood or serum from an infected patient. The HISG was ineffective when given intramuscularly or when mixed with the infective serum prior to its injection.

Holland et al.⁵¹ could not alter the incidence or severity of posttransfusion hepatitis in 84 open-heart surgery patients compared with 83 controls by giving HISG in two 10-ml doses 1 month apart. These findings were confirmed in a large cooperative study of 5,189 transfused cardiovascular patients given 10 ml of HISG during the first, fourth, and seventh postoperative weeks.⁵² In more recent studies, Redeker and associates⁵³ could not demonstrate that HISG protected spouses of individuals with type B hepatitis. Similarly, Kuhns et al.⁵⁴ could not reduce the incidence of post-transfusion hepatitis B with 20 ml of HISG. Both of these latter studies used HISG with low titers of antibody to the hepatitis B surface antigen (HBsAg).

These negative results are in contrast to several positive results. Torii et al.⁵⁵ reported that three cases of posttransfusion hepatitis developed in 18 patients given 20 ml or less of HISG, whereas no hepatitis developed in 63 transfused patients given 30 ml or more of HISG. Csapó et al.⁵⁶ noted only one case of hepatitis in 182 premature infants given 8 ml/kg of a 10% solution of HISG at the time of first blood transfusions, while 15 of 205 control infants who did not receive HISG developed hepatitis. Mirick et al.⁵⁷ noted that 10 ml of HISG given within 1 week of blood transfusion and again 1 month later reduced the incidence of icteric posttransfusion hepatitis from 3.89% to 1.07%. The incidence of anicteric hepatitis (6.3%) was not altered, indicating that the HISG modified some infections to anicteric and usually asymptomatic disease. Katz et al.⁵⁸ were able to reduce the incidence of icteric posttransfusion hepatitis from 0.89% of 2,019 patients to 0.25% of 1,970 patients by adding 10 ml of 6% hydrolyzed (modified) HISG to each unit of blood prior to transfusion. No reduction of anicteric hepatitis was observed.

Several factors are probably responsible for the variation of effectiveness of HISG in hepatitis B. One is the variable degree of exposure to the hepatitis B virus, which can be massive (as with a blood transfusion) or minimal (as with a casual sexual partner or household contact). A second factor is the variable level of hepatitis B antibody in various lots of HISG. It is now possible to measure the antibody content of HISG, usually by measuring anti-HBs; HISG lots with low titers are not protective.⁵³ Finally, HISG may be more effective in the prevention of other posttransfusion

hepatitis infections than in the prevention of posttransfusion hepatitis B. It is now recognized that only 20% to 30% of posttransfusion hepatitis is due to hepatitis B, as indicated by the presence of the HBsAg; presumably most are due to other viruses and have been termed non-A, non-B hepatitis, or hepatitis C.

Gerety et al.⁵⁹ showed that many (7% to 92%) of the HISG lots produced before 1972 had insignificant levels of anti-HBs antibody, but that since 1979 all tested HISG lots have anti-HBs titers of greater than 1:100 by RIA. These titers are significantly lower than those of hepatitis B immune globulin (HBIG), which has a titer of at least 1:100,000.

Perrillo et al.⁶⁰ compared the efficacy of HBIG and HISG following intimate (sexual) exposure to an index case of hepatitis B. Patients were given a single intramuscular injection of 0.06 ml/kg or 0.12 ml/kg of HISG or 0.06 ml/kg of HBIG. The anti-HBs titer of the HISG was 580 units, compared with a titer of 950,000 units in the HBIG. Despite these titer differences, all three regimens were equivalent. Four of 21 (19%) of the low-dose HISG group, three of 20 (15%) of the high-dose HISG group, and two of 19 (11%) of the HBIG group developed serologic evidence of infection. Two of the low-dose HISG patients developed clinical hepatitis, but none in the two other groups did. They suggest that current lots of HISG can be substituted for HBIG under certain circumstances, particularly if a higher HISG dose (0.12 ml/kg) is used.

Recommendations

Because of the availability of HBIG (discussed later), HISG is only occasionally used in the prevention of hepatitis B. HBIG or HISG is recommended following parenteral or mucous membrane (oral, sexual, ophthalmic) contact with individuals with hepatitis B infection or with HBsAg-positive materials (blood, plasma) and for neonates exposed to HBsAg mothers. When exposure is definite and/or extreme, HBIG is always the preferred product because of its higher anti-HBs titer.

Lots of HISG must have an anti-HBs of 1:100 by RIA; this titer is considered protective but is considerably less than the anti-HBs content of HBIG (at least 1:100,000). Because it is more widely available and considerably less expensive, HISG can be substituted for HBIG at equivalent or double doses under some circumstances. It is particularly valuable in situations in which the exposure is slight or unknown (e.g., exposure to a sexual partner, institutional exposure) or following exposure to a source that can be tested for HBsAg positivity. Under these circumstances, HISG (0.06 to 0.12 ml/kg) can be given immediately; if the source is proven to be HBsAg positive, HBIG is then given within 7 days and repeated at 30 days.

Because of the lower titer of anti-HBs in HISG, a double dose of HBIG (0.12 ml/kg, maximum 10 ml) rather than the usual HBIG dose (0.06 ml/kg, maximum 5 ml) is recommended.

HISG in Non-A, Non-B Hepatitis

In 1976 Knodell et al.⁶¹ showed that the incidence of icteric and nonicteric posttransfusion hepatitis could be reduced by administering either 10 ml of HBIG or HISG before cardiac surgery requiring multiple blood transfusions. Eight of 94 patients in the placebo-treated group developed icteric hepatitis, compared with one of 92 in the HBIG-treated group and one of 93 in the HISG-treated group. Since most of the patients who developed hepatitis had no serologic evidence of hepatitis B, cytomegalovirus (CMV), or Epstein-Barr virus infection and since HISG and HBIG were equally efficacious, they concluded that HISG (and HBIG) was reducing the risk of posttransfusion non-A, non-B hepatitis. Seeff et al.⁶² also noted that HISG (two 10-ml doses at 1-month intervals) reduced the incidence of posttransfusion icteric hepatitis from 7.1% to 2.9%; however, there was no reduction in the incidence or severity of hepatitis B.

In recent studies, Simon⁶³ was able to reduce the incidence of non-A, non-B hepatitis in hemodialysis patients by giving 5 ml of HBIG every 7 weeks. Non-A, non-B hepatitis affected 31 of 83 controls but only two of 67 HBIG recipients. Since none of the patients developed HBsAg, the protective effect of the HBIG was thought to be due to the presence of antibodies to non-A, non-B hepatitis virus. Sugg et al.⁶⁴ reduced the incidence of non-A, non-B hepatitis following cardiac surgery from 4.8% (ten of 208 cases in untreated controls) to 2.4% (five of 209 treated cases) by giving 15 ml of HBIG at the time of surgery.

Recommendations

Routine administration of HISG after blood transfusion to prevent hepatitis B or non-A, non-B hepatitis is not recommended. However, it might be considered following transfusion of a patient whose life would be threatened by the development of hepatitis. A dose of 0.12 ml/kg of HISG can be used.

HISG in Poliomyelitis

Before the development of poliomyelitis vaccines in the mid-1950s, HISG was used extensively in the prevention of poliomyelitis. Bodian^{65, 66} showed that Red Cross HISG had neutralizing antibody to all three strains of poliovirus in approximately equal titers and that rhesus monkeys given intramuscular poliovirus could be protected against disease by subcutaneous administration of HISG.

Bloxson,⁶⁷ in an uncontrolled study during a 1948 Texas epidemic, gave 841 contacts an average dose of 2 ml of HISG and noted only four cases at 1, 2, 3, and 42 days after the HISG injection. He suggested that the HISG was given too late to prevent the first three cases and that protection had worn off in the fourth case.

A committee on immunization of the National Foundation for Infantile

Paralysis recommended in March 1951 that a controlled study be conducted on the efficacy of HISG in the prevention of poliomyelitis during epidemics. Hammon and associates⁶⁸ subsequently undertook a massive field study in communities in three states during poliomyelitis epidemics. Fifty-five thousand children ages 1 to 11 received either HISG (average dose, 0.14 ml/lb) or gelatin in a double-blind fashion. During the first week after injection, 12 cases occurred among the HISG recipients and 16 cases occurred among the gelatin recipients. In the second week, there were three and 23 cases in the two groups, respectively, and in the third to fifth week there were six and 38 cases in the two groups, respectively. When protection was incomplete, there was some alteration of severity. Protection waned by 6 weeks and disappeared by 8 weeks. These clinical results were confirmed by virus isolation or rise in antibody titers in affected patients.

HISG is an inefficient method of poliomyelitis prophylaxis, preventing only one case for every 500 to 2,000 injections, and then only for a brief time. Its chief value was in close family contacts of affected children and in aborting severe local epidemics.

Recommendations

The use of HISG rarely is indicated in the prevention of poliomyelitis. An exposed unimmunized subject should be given 0.15 ml/kg of HISG. An unimmunized patient who is traveling to an endemic or epidemic area and who cannot have vaccine also can be given this dose of HISG for temporary protection.

HISG in Rubella

Rubella prevention by HISG is controversial because of its unreliable efficacy. Early studies such as those of Greenberg⁶⁹ in 1947 suggested some prophylactic benefit. Greenberg gave 20 children exposed to rubella 5 ml of HISG; none developed rubella, whereas six of 20 noninjected controls did develop rubella. Korns⁷⁰ in 1952 showed that one lot of HISG at a dose of 0.1 ml/lb partially protected mentally retarded institutionalized subjects against epidemic rubella. Nine of 45 HISG-injected subjects developed rubella compared with 35 of 60 uninjected controls, a significant difference. However, another HISG lot was ineffective and a third was only slightly effective, suggesting that their titers of rubella antibodies varied significantly.

Grayston and Watten⁷¹ studied the efficacy of HISG during a rubella epidemic in Taiwan in 1958. They found that 5 ml of HISG could reduce the incidence of clinical rubella from 20.4% to 8.5%. Lower doses were not effective. The period of protection lasted from 1 to 12 weeks after inoculation. There was no evidence of modification of infection. Brody et al.⁷² carried out similar studies during an epidemic in Alaska in 1964. HISG (0.55 ml/kg) resulted in an attack rate of 18% in school-age boys;

in uninjected girls the attack rate was 89%. Fifteen of 40 boys who were given HISG and who did not develop clinical rubella had a rise in antibody, indicating subclinical infection. The HISG protection lasted for 1 month.

Houser and Schalet⁷³ were able to prevent rubella completely with doses of 15 ml of HISG given to military recruits prior to exposure to rubella. Thus, passive immunity is best achieved by early administration of large doses.

Green et al.⁷⁴ and Krugman and Ward⁷⁵ conducted the most extensive studies on HISG prophylaxis in rubella, aimed at answering the question of its value in the prevention of congenital rubella syndrome when given to exposed expectant mothers in the first trimester of pregnancy. They deliberately exposed 200 children to rubella virus (as infected serum) by intramuscular injection or aerosol into the pharynx; this was, respectively, 100% and 80% effective in transmitting infection. HISG containing neutralizing antibody at titers of 1:32 to 1:64 given at doses of 0.12 to 0.2 ml/lb had no demonstrable effect in preventing rubella in experimentally inoculated subjects or in patients exposed to rubella. Viremia was not prevented, although the duration of viremia was shortened. The HISG dose was selected to approximate the 20-ml dose generally given to exposed pregnant women.

Lundstrom et al.⁷⁶ gave 251 exposed pregnant Swedish women 4 ml of convalescent rubella immune globulin and gave 28 exposed pregnant women 24 ml of standard HISG. Six of 251 (2.4%) developed rubella; three of these six women aborted, and one had a child with probable congenital rubella. None of the 28 women given 24 ml of HISG contracted rubella. They believed that this low incidence represented a significant protective effect. Their later studies demonstrated that convalescent rubella immune globulin given to women with manifest rubella did not protect against congenital rubella or lessen the probability of fetal damage.⁷⁷

Recommendations

The use of HISG to prevent rubella in males and nonpregnant females is not indicated because of the mildness of disease and the possible interference with active immunity.

HISG is not recommended in most nonimmunized exposed pregnant women, since the clinical syndrome may be masked and congenital rubella not reliably prevented. However, for those exposed pregnant women who for religious or other grounds will not consider therapeutic abortion, a large dose of HISG may be of some value. After possible exposure, an antibody test should be performed immediately. Several assays (latex or enzyme-linked immunosorbent assay [ELISA]) are available for rapid determination of rubella antibody. If antibody is present, the woman is not susceptible and she can be reassured. If antibody is absent or there will be a significant delay in obtaining the result, the woman is considered susceptible.

ble. Then standard HISG can be given in a total dose of 0.55 ml/kg. The antibody test is repeated after 1 month. A negative test indicates that rubella was prevented or exposure did not occur. A positive result (when the preinjection antibody test was negative) indicates that infection has occurred despite HISG. She can be counseled again regarding her risks of an infected fetus.

HISG in Varicella

Following the success of HISG in the prevention of measles and hepatitis, HISG was evaluated for the prevention of varicella. Although Funkhouser⁷⁸ in 1948 showed some beneficial effects of standard HISG in prevention of varicella in an uncontrolled study, Greenberg⁶⁹ and Schaeffer and Toomey⁷⁹ were unable to prevent chickenpox in exposed children using doses of 2.5 to 20.0 ml.

Others have presented anecdotal evidence for the efficacy of HISG in large doses during the early stages of chickenpox and herpes zoster. These claims include prompt relief of pain in zoster⁸⁰ and rapid resolution of skin lesions.^{81, 82} However, even high-titered zoster immune globulin does not prevent dissemination of herpes zoster.⁸³

Ross⁸⁴ in 1962 gave 242 children HISG in doses of 0.1 to 0.6 ml/lb within 3 days of exposure to chickenpox; 209 similarly exposed, uninjected children were used as controls. The attack rate was the same (97%) in both groups, indicating that HISG does not prevent varicella under these conditions. However, with doses of HISG above 0.2 ml/kg, the severity of the disease was reduced, as indicated by a decreased number of pox and lessened temperature. Children receiving the largest dose of HISG (0.6 ml/lb) had a mean maximal temperature of 38.9°C (102°F), compared with 41.1°C (106°F) for the controls and 40 pox versus 207 for the controls. Others have also reported similar but uncontrolled observations that HISG modifies the severity of chickenpox.^{85, 86}

Brunell and Gershon⁸⁷ subsequently showed that high-titered HISG from convalescing zoster patients could completely protect exposed normal children while standard HISG did not prevent the disease. Subsequent studies⁸⁸ have shown that high-titered immune globulin or plasma can modify or prevent chickenpox in high-risk children (e.g., leukemics). Standard HISG is not used in high-risk subjects.

Recommendations

For normal subjects exposed to varicella, standard HISG can be expected to modify the disease and varicella zoster (high-titered) immune globulin (VZIG) to prevent it.

Normal Children.—Since varicella is a mild disease in most children, prophylaxis is rarely indicated. The likelihood of prior chickenpox in the exposed individual, as discussed in the VZIG section, and the high cost of VZIG should also be considered. However, special circumstances (e.g., fu-

ture travel, scheduled surgery, risk of exposure of an immunocompromised sibling) may be indications for prophylaxis with HISG or VZIG.

Modification with HISG is achieved utilizing doses of 0.6 to 1.2 ml/kg. If prevention is sought, VZIG must be used. IGIV can also be used to prevent varicella (see VZIG section).

Adults.—Adults are more likely to develop severe varicella than are normal children (see VZIG section). Thus, the need to avoid or modify varicella is more compelling. Modification with HISG can be achieved by using a dose of 0.6 to 1.2 ml/kg with a maximum of 40 ml.

HISG in Miscellaneous Disorders

Asthma and Allergies

Several studies, reviewed by Thomas and McGovern,⁸⁹ have suggested a beneficial effect of HISG in asthma and other allergic conditions. Even 0.1-ml intradermal doses have been recommended⁹⁰; however, two double-blind studies could not find significant benefit from such therapy.^{91, 92} Thus, HISG should not be used in these disorders.

Acute Infections

Monthly HISG (0.15 to 0.4 ml/lb) was not beneficial in the prevention of upper respiratory infection, otitis, skin infection, gastrointestinal upset, or fever in children.⁹³ Finkel and Haworth⁹⁴ found that 0.4 ml/kg of HISG given to children younger than 2 years with acute respiratory infection was of no clinical benefit.

Premature Infants

Premature infants have significantly lower levels of IgG at birth, have a more severe and prolonged period of physiologic hypogammaglobulinemia, and are more susceptible to infectious disease and sudden infant death syndrome. Further, they have a transient opsonic deficiency, partly correctable with HISG.⁹⁶ Thus, the use of HISG or IGIV in the prophylaxis of infection in these infants has theoretic justification.

Amer and colleagues⁹⁷ gave HISG monthly (1.5 ml/kg) for 8 months to 92 premature infants and gave albumin to 69 control premature infants. There were six infectious deaths in the albumin-treated group and one infectious death in the HISG-treated group. HISG did not affect the incidence of severe infections but did decrease the overall frequency of infections (43.5% vs. 85.3%) and the length of hospital stay (7.8 days vs. 12.4 days). There was a slight decrease of mean γ -globulin levels in the treated infants (620 mg/dl vs. 720 mg/dl), either as a result of increased infection in the control group or suppression of IgG synthesis by the passive HISG in the treated group.

These results, as well as other studies that show no beneficial effect of HISG in premature infants,^{98, 99} indicate that HISG is of unproven value and is not indicated in the routine management of low-birth-weight infants.

Burns

Kefalides et al.¹⁰⁰ were able to reduce the mortality rate of severely burned children (10% to 30% of surface area) by administering plasma (1 ml/kg body weight for each 1% of surface area burned) or HISG (1 ml/kg on days 1, 3, and 5) from 40% to 20%. They concluded that substances that contain antibodies (plasma or HISG) were more effective in reducing infection complications than were other colloids. However, Stone et al.¹⁰¹ could not achieve any clinical benefit from HISG therapy (0.4 ml/kg every third day until skin coverage) in 60 burned subjects compared with 40 controls.

Convalescent plasma, special HISG with high antibody titer to *Pseudomonas*, and *Pseudomonas* vaccines have also been used in burn patients in attempts to reduce infections but without proof of efficacy.^{102, 103} IGIV has also been used.

Malaria

Cohen et al.¹⁰⁴ showed that hyperimmune HISG from convalescing adults given to young children 4 months to 2.5 years of age in doses of 1.2 to 2.5 gm significantly reduced their blood trophozoite count, compared with untreated patients or patients treated with HISG from normal adults. These children remained protected for a period of 3 months, at which time they were susceptible to reinfection. These studies suggest that humoral antibody exerts a beneficial effect in malaria and that a vaccine against malaria may be efficacious.¹⁰⁵

Severe Bacterial Infections

Animal studies suggest that HISG has some value in the treatment of staphylococcal and *Pseudomonas* infections; further, when used with antibiotics such as chloramphenicol and penicillin, the combination is more effective than antibiotics or HISG alone.¹⁰⁶ These studies may not be analogous to the clinical situation since the HISG is usually given before, concomitantly, or shortly after onset of experimental infection. Numerous case studies suggest that in certain refractory infections, the addition of HISG to the antibiotic regimen has provided some therapeutic benefit. In some, there is dramatic improvement following the addition of HISG to a long, unsuccessful course of antibiotic therapy.¹⁰⁷ Waisbren¹⁰⁸ found therapeutic benefit of HISG in 6 of 46 patients with refractory infections, most due to *Staphylococcus*. These patients, none of which had hypogammaglobulinemia, were given HISG in doses of 0.7 to 1.0 ml/kg.

Bodey et al.¹⁰⁹ could not demonstrate a therapeutic benefit of HISG in the treatment of infection in acute leukemia. All patients received antibiotics. Many of the fevers were unexplained and may have been due to the underlying disease. With the availability of IGIV, this problem is being re-studied using higher doses of γ -globulin.

Ziegler and associates¹¹⁰ used a human antiserum to *Escherichia coli*

endotoxin in the treatment of gram-negative bacteremia. The death rate in untreated bacteremic patients was 39% (42 of 109) compared with 22% (23 of 103) in recipients of antiserum. In those with profound shock, mortality was 77% (30 of 39) in controls and 44% (18 of 41) in recipients of antiserum, a significant difference. Both control and antiserum groups received similar antibiotics and had similar microbiologic causes for their infections. This suggests that a specifically titered human IgG to endotoxin would be of therapeutic value.

Use of Special Human Immune Serum Globulins

Preparation of the special human immune serum globulins (see Table 3) is identical to that of HISG except that the donor pool includes subjects immunized to or convalescing from a specific illness who have a high titer of antibody to the microbial agent. Thus, the pharmacologic properties of standard HISG and special HISGs are identical. The only difference is that the antibody titer of the special HISG to the specific virus, bacterium, or toxin is assayed to assure a potent preparation.

Hepatitis B Immune Globulin

HBIG has been licensed since 1978 for the prevention of hepatitis B.¹¹¹ It is derived from donor lots of HISG that have titers of anti-HBs of at least 1:100,000 by RIA.

Krugman et al.¹¹² in 1971 first evaluated high-titered HBIG in institutionalized children injected with the infective serum MS-2. All 11 children exposed to MS-2 serum developed hepatitis; two became icteric and five remained HBsAg carriers after 320 days. Among five children given MS-2 serum and standard HISG, three developed hepatitis; two were icteric, but none became a carrier. Among ten children exposed to MS-2 serum and HBIG, six were completely protected, one had a transient infection, and four developed classic hepatitis. They concluded that HBIG was 70% effective under these circumstances. Their later studies confirmed that HBIG could significantly reduce the incidence, severity, and carrier rate of HBsAg following parenteral exposure to HB virus.⁴⁵

Szmuness et al.¹¹³ tested the efficacy of HBIG compared with standard HISG by giving either HISG or HBIG to retarded institutionalized children at admission and at 4-month intervals for 1.5 to 2.0 years and comparing the incidence of hepatitis to that of untreated subjects. Both globulin-treated groups had a lower attack rate (11% vs. 25%) and lower incidence of chronic antigenemia (0% vs. 13.5%). Thus, both HISG and HBIG may be effective in preventing or modifying nonparenterally transmitted hepatitis B in an endemic setting. Of note is that 55% of the patients treated with standard HISG developed anti-Hbs, whereas only 23% of the patients treated with HBIG developed antibody, suggesting that passive-active im-

munity occurred more frequently in the group that received standard HISG.

Seeff et al.¹¹⁴ gave either HBIG or standard HISG to 302 individuals accidentally exposed to material infectious for hepatitis B. The incidence of clinical or subclinical hepatitis during the first 6 months was 0.7% in the HBIG-treated group and 6.1% in the HISG-treated group. At 6 months, 32% of the HISG recipients and 6% of the HBIG recipients had antibody, indicating minimal passive-active immunity in the HBIG-treated group; Grady et al.¹¹⁵ reported similar results with HBIG after accidental exposure. The incidence of hepatitis at 6 months was 7% (of 251 patients) with standard HISG, 5% (of 208 patients) with intermediate-titer HBIG, and 2% (of 253 patients) with high-titer HBIG. This protection waned after 6 months, and differences in the groups became less apparent after 9 months, possibly due to re-exposure, delayed onset of infection, or failure of passive-active immunity.

Two studies in renal dialysis units also support the effectiveness of HBIG. Desmyter et al.¹¹⁶ found that HBsAg antigenemia occurred at 16 months in ten of 14 dialyzed patients given standard HISG every 6 months, but in only two of 15 dialyzed patients given HBIG on a similar schedule. Prince et al.¹¹⁷ gave standard HISG or HBIG to 318 new dialysis patients and 296 staff members every 4 months; they reduced the incidence of hepatitis B from 23.1% in the HISG patients to 7.9% in the HBIG patients. There was only a slight decrease in incidence of hepatitis in the HBIG-treated staff members (11.1% vs. 6.9%).

Redeker et al.¹¹⁸ found that HBIG was effective in preventing hepatitis B after 150 days' exposure in spouses of patients with hepatitis B. Nine of 33 spouses treated with standard HISG developed hepatitis, whereas only one of 25 spouses receiving HBIG developed hepatitis B.

Prevention of Vertical Transmission From Mothers to Infants

Beasley et al.^{119, 120} studied the efficacy of HBIG in preventing perinatal transmission of the hepatitis B virus carrier state from a mother to her newborn infant. HBIG or placebo was given at birth to infants of the hepatitis B e antigen (HBeAg)-positive, HBsAg carrier mothers, and the infants were followed for at least 15 months. Among 61 placebo recipients, 92% became carriers; among 67 infants who received 0.5 ml of HBIG at birth or in addition at 3 and 6 months, 26% became carriers. Passive-active immunization, indicated by the presence of anti-HBs, occurred in 27% of the single-dose group.

Based on this and other studies^{121, 122} that suggested that multiple HBIG doses were more effective in interrupting vertical transmission of the HBsAg carrier state than a single HBIG dose, advisory committees in 1981 recommended that all infants of HBsAg-positive mothers be given HBIG (0.5 ml) immediately after birth and again at 3 and 6 months. However, a number of these infants became infected some time after their last HBIG

dose (i.e., in the second or third year of life), indicating a need for more durable active immunity.

Wong and associates¹²³ studied the efficacy of hepatitis B vaccine (HBV) given in conjunction with HBIG in the prevention of vertical transmission of the carrier state from mother to infant. They gave HBV (36 infants), HBV plus one dose of HBIG (35 infants), HBV plus seven monthly HBIG doses (35 infants) or placebo (35 infants) to infants of HBsAg-positive mothers. In all vaccine groups, development of the persistent carrier state was significantly reduced compared with the placebo group (21%, 2.9%, and 6.8%, respectively, vs. 73.2% in the placebo group). All infants of the treatment groups developed anti-HBs indicating that HBIG did not interfere with active immunization.

This and other studies¹²⁴⁻¹²⁶ indicate that HBIG given at the time of birth followed by HBV provides optimal passive-active immunity for long-lasting prevention of the carrier state, and this is the current recommendation. Studies in adults also confirm that HBIG given before or simultaneously with the first dose of HBV does not interfere with the antibody response to HBV.^{127, 128}

HBIG is not of value in the treatment of either acute¹²⁹ or chronic¹³⁰ hepatitis B infection.

Recommendations

HBIG is recommended following parenteral or mucous membrane (oral, sexual, ophthalmic) contact with individuals with hepatitis B infection or with HBsAg-positive materials (e.g., blood, plasma) and for neonates born to HBsAg-positive mothers (Table 6).

Exposure to Blood Products Containing HBsAg.—There are no prospective studies testing the efficacy of a combination of HBIG and HBV in preventing hepatitis B after accidental exposure. This includes exposure by the percutaneous, ocular, and mucous membrane routes as well as by human bites that penetrate the skin. Since health care workers at risk from such accidents are HBV candidates and since combination HBIG and HBV is more effective than HBIG alone in perinatal exposure, this combination is also recommended following accidental exposure.

If the blood or secretions come from an individual known to be HBsAg positive or if the status of the exposure donor is unknown, immediate prophylaxis is indicated. A single dose of HBIG (0.06 ml/kg or 5 ml for adults) should be given as soon as possible, preferably within 24 hours of exposure. HBV (1 ml) should be given simultaneously at a different site and repeated after 1 and 6 months.

After massive exposure (i.e., via a blood transfusion), much larger doses of HBIG are probably indicated. If HBIG is unavailable, HBIG may be given at an equivalent or double dose (at least 0.12 ml/kg or 10 ml for adults).

If an individual has received at least two doses of HBV before accidental exposure, HBIG is unnecessary if serologic tests show adequate anti-HBs

TABLE 6.
Hepatitis B Virus Postexposure Recommendations*

HBIG			Vaccine	
Exposure	Dose	Recommended Timing	Dose	Recommended Timing
Perinatal	0.5 ml IM	Within 12 hours of birth	0.5 ml (10 µg) IM	Within 7 days;† repeat at 1 and 6 months
Percutaneous	0.06 ml/kg IM or 5 ml for adults	Single dose within 24 hours or§	1.0 ml (20 µg) IM‡	Within 7 days;† repeat at 1 and 6 months
Sexual	0.06 ml/kg IM or 5 ml for adults	Within 24 hours; repeat at 1 month
	0.06 ml/kg IM or 5 ml for adults	Within 14 days of sexual contact		...

*From Centers for Disease Control: Postexposure prophylaxis of hepatitis B. *MMWR* 1984; 33:285-290.

†The first dose can be given the same time as the HBIG dose but at a separate site.

‡For persons less than 10 years of age, use 0.5 ml (10 µg).

§For those who chose not to receive hepatitis B vaccine.

||Vaccine is recommended for homosexually active males and for regular sexual contacts of chronic hepatitis B virus carriers.

titers (greater than 10 units by RIA). If HBV is not given, two doses of HBIG should be used, the second given 1 month after the first.

Perinatal Exposure.—If the mother is HBsAg and HBeAg positive, 85% of her offspring will become infected and will become chronic carriers, some of whom will develop chronic hepatitis, cirrhosis, or hepatic cancer. If the mother is HBsAg positive only, the risk of her offspring becoming carriers is less, but still significant. Accordingly, these infants are also candidates for prophylaxis.

For optimal passive-active immunity, HBIG (0.5 ml) is given to the newborn at birth (preferably in the delivery room but within 12 hours of birth at the latest). HBV (0.5 ml) is begun simultaneously and repeated at 1 and 6 months (see Table 6). This combination is only about 90% effective in preventing the carrier state, since intrauterine infection will not be prevented.

HBIG effectiveness is markedly diminished if administration is delayed beyond 48 hours. Nevertheless, if the mother is found to be HBsAg positive at birth, HBIG and HBV should be given to her infant even if there has been a significant delay. The infant should be tested for HBsAg and anti-HBs at 12 to 15 months to determine the success of the HBIG and HBV regimen. If HBsAg is present, the infant probably is a carrier; if HBsAg is absent and anti-HBs is present, the child has been successfully immunized. HBIG administration at birth should not interfere with polio or diphtheria-pertussis-tetanus (DPT) vaccines given at 2 months of age.

Sexual Exposure.—Sexual exposure to an individual who has, who develops, or who is a carrier of hepatitis B is an indication for HBIG (0.06 ml/kg, 5 ml maximum). This should be effective for about 14 days after the exposure. If the contact's HBsAg status is not known, it should be determined; if he or she is HBsAg positive, HBIG should be given. If sexual contact continues and if the contact remains HBsAg positive for more than 3 months, a second HBIG dose is recommended.

HBIG should also be given if sexual contact with HBsAg-positive individual is anticipated. Contacts who remain HBsAg positive are probably carriers, and HBV should be given to their regular sexual partners.

For sexual exposures among homosexual men, HBIG is given and HBV should be given simultaneously; indeed, HBV is recommended for all susceptible sexually active homosexual men. Additional doses of HBIG are unnecessary if HBV is given.

Possible Exposures.—Following possible exposures (percutaneous, ingestion, sexual) to an unidentified person or body fluid in which the HBsAg status is unknown, a decision to treat with HBIG (or HSiG) must be made individually, based on the likelihood that the source is HBsAg positive and the seriousness of the exposure.

Ideally the source should be tested for HBsAg positivity; if the results are available within 7 days, HSiG (0.06 ml/kg) can be given immediately, followed by HBIG at 7 days (0.06 ml/kg, 5 ml maximum) and again at 1 month if the source is HBsAg positive. When the source cannot be tested

or when the source is likely to be HBsAg positive, HBIG is given immediately and again at 1 month.

If the exposed individual is a high-risk patient (e.g., immunodeficient, immunosuppressed, institutionalized Down's syndrome, Asian, or undergoing hemodialysis) or is in a unit for which past environmental control measures have been unsuccessful (e.g., a mental institution, hemodialysis unit) or is a member of the staff of such units with considerable patient contact, he or she is also a candidate for prophylaxis.

HBIG or HISG is not indicated on a routine basis following blood transfusions. A school or hospital exposure is not an indication for HBIG or HISG.

Pertussis Immune Globulin

In 1917 Bleyer¹³¹ used convalescent blood in the treatment of whooping cough but could demonstrate no therapeutic benefit. Debre⁴ in 1923 successfully used convalescent serum during the incubation period to prevent or modify the disease. French workers subsequently conducted studies that suggested that convalescent blood or serum given early in the incubation period results in complete protection, given late in the incubation period results in modification, and given during the coughing stage is ineffective.¹³² Jundell¹³³ in 1934 first reported on the beneficial use of immune serum from vaccinated adults in the treatment of pertussis in young infants. In 1940, McGuinness et al.¹³⁴ used lyophilized hyperimmune whooping cough serum (from repeatedly vaccinated donors). The disease was prevented in 65 and markedly attenuated in 11 of 83 children exposed to pertussis; it also had some beneficial effect when given to children with early pertussis.

Felton¹³⁵ reviewed the overall experience with hyperimmune serum. These uncontrolled studies suggested that hyperimmune serum given in large doses early in the incubation period was moderately effective (75%) in preventing pertussis and given early during the disease was of some benefit in modifying pertussis. The patients benefiting most were infants under 1 year of age; large intravenous doses were necessary for these critically ill infants.

Kohn and associates¹³⁶ in 1947 reported on the use of hyperimmune rabbit serum in the early treatment of pertussis but felt that hyperimmune human serum was more efficacious. Lucchesi and LaBocchetta¹³⁷ conducted a controlled study of 52 antibiotic-treated infants under 1 year of age and noted that hyperimmune human serum given during the first week reduced slightly the frequency of paroxysms. Ames et al.¹³⁸ in 1953 compared the efficacy of streptomycin, chloramphenicol, rabbit antiserum, and human antiserum, in addition to penicillin, in the treatment of pertussis. They found that most cases of pertussis are mild if secondary bacterial infections are treated promptly with penicillin and that all four therapeutic regimens were equally efficacious in modifying the course of the disease.

Rabbit antiserum was the most effective in eliminating the pertussis organism. Thus, with the advent of antibiotic therapy, the need for passive immunization was considerably lessened.

Morris and McDonald¹³⁹ in 1967 could find no protective effect of human hyperimmune pertussis immune globulin in the prevention of pertussis in family contacts. Bass and associates¹⁴⁰ could not demonstrate any additional therapeutic effect of pertussis immune globulin given to 17 antibiotic-treated children compared with 23 children given antibiotics alone. In a double-blind study of 127 children with pertussis, Eichlseder¹⁴¹ could not show that pertussis immune globulin, chloramphenicol, or the combination affected the frequency of coughing, the duration of the paroxysmal phase, or the pulmonary complications. Balagtas et al.¹⁴² in 1971 conducted a double-blind study of the efficacy of 2.5 ml of pertussis immune globulin in 40 patients and 36 controls. All patients received ampicillin. No differences were observed in the rate of recovery; the frequency of paroxysms, coughing, or whooping; number of episodes of vomiting; the need for suctioning; or frequency of pulmonary complications.

In sum, passive immunization was of clinical benefit in the prevention and early treatment of pertussis prior to the availability of effective antibiotics. When used in conjunction with optimal antibiotic therapy, pertussis immune globulin provides little if any additional therapeutic benefit.

Recommendations

There are no absolute indications for the use of pertussis immune globulin, providing antibiotics are available. In young, very ill infants in whom antibiotics are not controlling the disease, pertussis immune globulin could be given in an attempt to augment the body's natural defenses. The dose is 1.25 to 2.5 ml intramuscularly daily for 3 days.

Rabies Immune Globulin

Rabies is the ideal disease for passive immunization since the exact moment, the exact source, and the exact location of exposure are known. Further, the long incubation period and that the virus remains localized to the wound for several days enhance the effectiveness of passive immunization.

Rabies serum was first prepared in 1889 by Babes and Lepp.² However, variable experimental results, difficulty in interpreting field results, and the development of rabies vaccine led to a loss of interest in passive immunity. Habel¹⁴³ in 1945 conducted a series of studies of experimental rabies in mice, guinea pigs, and monkeys using rabbit hyperimmune serum. He showed that antibody worked by two mechanisms: (1) it neutralizes the virus while still in the tissues and (2) it retards the spread of virus to the central nervous system, thus prolonging the incubation period and permitting active immunity by vaccine. Serum prophylaxis alone gave consistently better results than vaccine alone, was effective when given up to 3

days after infection, and was more effective when given locally at the site of virus inoculation.

On the basis of these studies, a World Health Organization Expert Committee in 1950 recommended that a field trial of the efficacy of hyperimmune rabies serum, in conjunction with vaccine, be conducted.¹⁴⁴ This was undertaken in Iran since multiple bites by a single rabid wolf coming into isolated villages were common and this severe exposure had an associated 40% to 50% mortality. In 1954, a single rabid wolf bit 27 individuals, 17 of whom were bitten on the head. These 17 were divided into three groups: five received vaccine alone, seven received vaccine and one dose of antirabies serum, and five received vaccine and two doses of antirabies serum.¹⁴⁵ Three of five persons treated with vaccine alone died of rabies, one of seven in the one-dose antiserum group died, and none of five in the two-dose antiserum group died. Antibody studies conducted on these patients indicated that a single or a double dose of antiserum, followed by 14 to 21 daily doses of vaccine, results in significant levels of circulating antibody for as long as 50 days.¹⁴⁶ The antibody found early is supplied passively; after the tenth day, the antibody present is a result of the vaccine. Thus, optimal treatment requires both passive and active immunization.

Prior to 1971, the available antiserum was of equine origin. Since then, human rabies immune serum also has been available and is preferred because of the lessened risk of serum reactions.¹⁴⁷ Further, the human antibody has a half-life in the circulation twice that of equine antibody, with the result that higher levels of passive antibody are maintained. However, the antibody response to the vaccine given concomitantly is more effectively suppressed.¹⁴⁸ Accordingly, smaller quantities of human rabies immune globulin (RIG) must be given to achieve passive-active immunity (20 IU/kg); when larger doses (40 IU/kg) are given, significant depression of active immunity may result.¹⁴⁹

Since 1980 the vaccine of choice is human diploid cell rabies vaccine; duck embryo vaccine is no longer available in the United States. The improved vaccine does not lessen the need for simultaneous passive immunity at the time of exposure; indeed cases¹⁵⁰ of fatal rabies have been reported following rabies exposure in which diploid cell vaccine but no RIG was given. A single case has been reported of fatal rabies encephalitis following an animal bite despite prompt use of diploid vaccine and RIG.¹⁵¹

Recommendations

RIG or rabies antiserum is recommended for all bites by animals in which rabies cannot be ruled out and for nonbite exposure to animals proved or suspected of being rabid.¹⁵² Such treatment should be given as early as possible after exposure but should be used regardless of the interval between exposure and treatment. Half of the RIG (or equine rabies antiserum) should be injected locally at the wound site and the rest given intra-

muscularly. Vaccines should then be given at a different site with a different needle and syringe.

When both preparations are available, human RIG is preferred because of the decreased risk of serum reactions. If RIG is unavailable, equine rabies antiserum should be used. If an individual is known to be allergic to horse serum, RIG must be used or a schedule of desensitization employed.

The recommended dose is 20 IU/kg of RIG or 40 IU/kg of equine rabies antiserum. RIG (or antiserum) is given as soon after exposure as possible. If RIG inadvertently was not given at the time of exposure, it should be given immediately regardless of the time elapsed. If vaccine has been started, RIG should still be given for up to 8 days after the first dose of vaccine.

RIG is supplied in 2-ml and 10-ml vials containing 150 IU/ml. Equine rabies antiserum is supplied in 5-ml vials containing 200 IU/ml. Since RIG and rabies antiserum may inhibit the immune response to simultaneously administered rabies vaccine, it is necessary to give five doses of vaccine rather than the three recommended for preexposure primary immunization given alone. Following exposure to possible rabies, rabies vaccine is recommended at 0, 3, 7, 14, and 28 days when RIG is given at day 0. Antibody testing should be done 2 to 3 weeks after the last injection to assure an adequate antibody response.

RIG is not used simultaneously when rabies vaccine is given prophylactically to unexposed high-risk individuals.

RIG and rabies antiserum are of no value in the treatment of established rabies infection.

Tetanus Immune Globulin

Antitoxin in the treatment of tetanus was introduced in medicine by Behring and Kitasato¹⁵³ in 1980; large doses (50 to 100 ml) of serum from horses immunized with tetanus toxin were used. The dose was gradually increased to 300 to 500 IU of antitoxin. As means to increase the production and concentration of antitoxin developed and a high mortality rate persisted, the dosage of antitoxin was increased until doses as high as 200,000 IU, repeated at weekly intervals, were recommended.¹⁵³ Despite such heroic therapy, there was no solid proof of efficacy.

In 1960, Brown and colleagues,¹⁵⁴ using sequential analysis, found the mortality rate was 49% among 41 tetanus patients receiving 200,000 IU of antitoxin and 76% in 38 patients not receiving antitoxin, a statistically significant difference. In controlled studies, there was no improvement in mortality rates when 5,000 to 500,000 IU were used.^{153, 155-159} Adequate blood levels of antitoxin were noted in all cases with a dose of 10,000 units. Considerable differences were noted in mortality rates, ranging from 0% to 98%; this primarily depends on the severity rather than the dose of antitoxin.

Athavale et al.¹⁶⁰ established that antitoxin was of benefit in tetanus

neonatorum and in tetanus in children up to age 12 in mild and moderate cases but not in severe cases. A dose of 10,000 IU was as effective as 30,000 IU.

Antitoxin neutralizes toxin prior to its arrival in the nervous system via the circulation or locally and prevents its systemic absorption. Thus, antitoxin should be given at the site of toxin production (e.g., at the site of a wound) and intravenously (in severe cases) or intramuscularly, the latter being reserved for less severe cases.

It was estimated that 750,000 annual doses of tetanus antitoxin (TAT) were needed in the United Kingdom, or 2 million doses in the United States.¹⁶¹ Serum sickness occurs in 6% to 14% and fatal anaphylaxis, in one of every 100,000 injections. Thus, hyperimmune human tetanus immune globulin (TIG), first available in the early 1960s, has gradually replaced equine TAT.

Rubbo and Suri¹⁶² and Rubinstein¹⁶¹ showed that human TIG given intramuscularly (5 to 10 units/kg) provides adequate circulating antitoxin levels and is maintained in the circulation for a considerably longer time than is equine TAT.

The efficacy of human TIG is equivalent to that of equine TAT. McCracken et al.¹⁶³ compared the results of 500 units of TIG with 10,000 units of TAT in the treatment of tetanus neonatorum. Among the 65 infants in each treatment group, there was no difference in severity, length of hospital stay, need for sedation or gavage feeding, or mortality (43% and 42%, respectively). Blake et al.¹⁶⁴ analyzed 545 tetanus cases reported to the Center for Disease Control from 1965 to 1971 and could find no difference in outcome between those patients treated with equine TAT or TIG.

Gupta et al.¹⁶⁵ gave TIG intrathecally to alternate patients with early tetanus. Among 49 patients given intrathecal TIG (250 IU), three got worse and one died; among 48 patients given intramuscular TIG (1000 IU), 15 got worse and ten died. There were no side effects.

TIG can be given along with tetanus toxoid (10 Lf units) for passive-active immunization. A dose of 250 IU of TIG given intramuscularly at a site different from that of the toxoid does not interfere with the active antibody response.¹⁶⁶

Recommendations

Prophylaxis.—If a nonimmunized individual sustains a serious injury or a bite, 250 to 500 IU of TIG should be given intramuscularly. The larger dose should be used if there is an extensive wound or delay in treatment. Alum-precipitated toxoid to initiate active immunity is given at a different site, using a separate syringe. If TIG is unavailable, 3,000 to 5,000 units of TAT (bovine or equine) are given (after screening and testing the patient for serum sensitivity).

Treatment.—In addition to antibiotics and wound management, TIG

in doses of 500 to 3,000 IU should be given, part infiltrated near the wound and the rest given intramuscularly.

If TIG is unavailable, equine TAT should be given in a single dose of 50,000 to 100,000 units, with 20,000 units given intravenously (after appropriate testing for sensitivity). In severe cases, up to 50,000 units of TAT should be given intravenously. Intrathecal TIG or TAT is usually unnecessary. Upon recovery, primary immunization should be undertaken. In tetanus neonatorum, McCracken et al.¹⁶³ found that 500 IU of TIG intramuscularly or 10,000 units of equine antitoxin were equally efficacious.

Vaccinia Immune Globulin.—Although smallpox has been eradicated from the globe since 1979, the virus exists in a few research laboratories throughout the world, and vaccination is still being used on a limited basis (including the US military). Passive immunization is occasionally necessary following laboratory accidents, after inadvertent vaccination of high-risk individuals, or after exposure of high-risk individuals to recent vaccinates.

Passive immunization against vaccinia was known as early as 1895, when Hlava and Honl¹⁶⁷ showed that school children could be protected from vaccinia by the injection of 3 to 10 ml of immune calf serum. Protective antibodies following vaccination and passive transmission of these antibodies from mothers to infants were demonstrated.¹⁶⁸ Thereafter, the effective prevention of smallpox by well-organized mass vaccine campaigns diminished interest in passive immunization.

Janeway, quoted by Enders,¹⁶⁹ in 1944 found neutralizing vaccinia antibody in HISG, and Verlinde and Spaander¹⁷⁰ in 1966 found high titers of such antibodies in convalescent immune globulin from recently vaccinated individuals. Gispén et al.¹⁷¹ in 1956 developed a human vaccinia immune globulin (VIG) that was shown not to interfere with active immunity, and they proposed its use with vaccine as prophylaxis against vaccinia encephalitis.

The value of VIG in smallpox or disseminated vaccinia is based on the presence of viremia, which leads to secondary dissemination; administration of neutralizing antibody will prevent or limit the spread of infection and thus modify the clinical expression of the disease.

Studies on the efficacy of VIG in smallpox and vaccinia complications was initiated by Kempe and colleagues in 1955.¹⁷²⁻¹⁷⁵ VIG prepared from recently vaccinated donors had a neutralizing titer of 1:256 to 1:512, considerably higher than the titer (1:16 to 1:32) present in standard HISG. The households of new admissions to the Madras (India) Smallpox Hospital were visited, and alternate family contacts received VIG (1.0 gm in adults, 0.5 gm in children).¹⁷⁴ After 25 days, eight cases of smallpox developed in 75 contacts not given VIG and two cases in 56 contacts given VIG. A more extensive study disclosed 21 cases of smallpox (four severe) among 379 contacts serving as controls and five cases of smallpox (none severe) among 326 contacts given VIG.¹⁷³

Kempe¹⁷⁵ also reported the results of 300 cases of smallpox vaccination

(vaccinia) complications treated with VIG; these included 62 cases of generalized vaccinia, 132 cases of eczema vaccinatum, 23 cases of vaccinia necrosum, 12 cases of vaccinia encephalitis, and 28 cases of autoinoculation. In addition, VIG was given prophylactically to 44 eczematoid children requiring smallpox vaccine (0.6 to 1.2 ml/kg). VIG did not affect the course of vaccinia encephalitis. Twenty-seven of 28 patients with autoinoculation who received VIG did well. There were nine deaths among 132 patients with eczema vaccinatum given VIG; this 7% mortality compares favorably with the usual mortality of 30% to 40% with supportive care only. All 62 patients with generalized vaccinia given VIG did well, although four children required a second course. Among 23 patients with vaccinia necrosum who received VIG, there were seven deaths (30%); however, this is a generally fatal disease and immune defects were present in most of these patients.¹⁷⁵ These results strongly supported the efficacy of VIG and were subsequently confirmed by studies in Sweden¹⁷⁶ and the United Kingdom.¹⁷⁷

Nanning¹⁷⁸ studied the effect of VIG on postvaccinia encephalitis. He gave a placebo or 2 ml of VIG to Dutch military recruits at the time of primary vaccination; there were three cases of encephalitis among 43,630 vaccinated recruits given VIG, compared with 13 cases among 53,044 recruits in the control group.

Recommendations

Nonimmunized patients exposed to smallpox virus should be given VIG intramuscularly at a dose of 0.3 ml/kg 12 to 24 hours after smallpox vaccine.¹⁷³ VIG is no longer manufactured in the United States but is available from the US Centers for Disease Control in Atlanta.

Eczematous children who must be vaccinated should be given VIG (0.3 ml/kg) intramuscularly simultaneously at another site. Eczematous children or other individuals with extensive dermatitis inadvertently exposed to a recently vaccinated individual should also receive VIG (0.3 ml/kg).

Accidental vaccination or autoinoculation of the eye, eczema vaccinatum, severe generalized vaccinatum, severe generalized vaccinia, and vaccinia necrosum are all indications for VIG. The initial dose is 0.6 ml/kg, but repeat doses may be necessary. If VIG is not available, standard HISG can be used in larger doses. The material has about one fourth the potency of VIG.

VIG is not indicated for established smallpox infections, postvaccinia encephalitis, or hypersensitivity and toxic rashes following vaccination.

Varicella-Zoster Immune Globulin

The prophylactic value of large HISG doses in decreasing the severity of varicella led to the trial of high-titered plasma or γ -globulin preparations in the prevention of varicella. These preparations include zoster immune globulin (ZIG) and zoster immune plasma (ZIP) from convalescing zoster patients and VZIG prepared from high-titered normal adult sera.

Brunell et al.¹⁷⁹ in 1969 selected convalescing zoster patients whose complement-fixing antibody titers were 1:256 or greater and prepared ZIG from their plasma; this material had titers considerably higher than did standard HISG. Exposed children from six families in which chickenpox was occurring were given ZIG or HISG at doses of 2 ml. None of six children receiving ZIG whereas all six children given HISG developed chickenpox. No antibody developed in the ZIG-treated group, indicating that the disease was prevented.

Since this dose did not prevent varicella in leukemic children or other high-risk patients, a larger dose (5 ml) was used in a later study to successfully modify or prevent varicella in eight of nine high-risk children.⁸⁸ Severe varicella developed in one child given a less potent preparation of ZIG. Judelsohn et al.¹⁸⁰ gave ZIG to 56 exposed high-risk children; mild varicella occurred in seven and was prevented in the others, most of whom are susceptible as determined by absence of serum antibody. Gershon et al.¹⁸¹ gave ZIG to 15 seronegative high-risk exposed children; varicella was severe in one, mild in nine, and subclinical in five. Subclinical infection was determined by the acquisition of membrane antibody, detected by fluorescent microscopy. Orenstein et al.¹⁸² studied 553 exposed, high-risk patients who received ZIG of two different titers (1:1280 vs. 1:2560 or greater). They found that the clinical attack rate after ZIG was correlated with the type of exposure (36% with a household exposure, 7.7% with a hospital exposure, and 0% with a school exposure developed varicella), the rise in antibody titer (45% of patients without a fourfold titer increase became ill compared with 22% of patients with a fourfold or greater rise in titer), and the titer of the administered ZIG (there were significantly more complications and deaths among recipients of the lower-titer ZIG).

Because of a limited supply of ZIG and ZIP, VZIG from normal adults has become the commercially available product in the United States. Zaia and associates¹⁸³ compared the efficacy of ZIG and VZIG in immunocompromised children exposed to varicella. The varicella attack rates and clinical severity of recipients of VZIG and of ZIG did not differ significantly. There was a higher incidence of subclinical infection as indicated by a rise in antibody titer in the ZIG recipients (31.3%) than in the VZIG recipients (16%). A larger dose of VZIG (2.5 ml/10 kg vs. 1.25 ml/kg) reduced the frequency of subclinical infection (from 20% to 4.3%). Several high-risk patients with demonstrable serum antibody at exposure developed varicella, indicating that history-negative seropositive patients are at risk for clinical varicella-zoster infection and should be given VZIG regardless of antibody titer.

Paryani and associates in 1984¹⁸⁴ observed that eight patients given IGIV at standard doses (200 to 300 mg/kg) achieved varicella-zoster virus antibody titers comparable to those achieved by individuals given intramuscular VZIG at standard doses, despite the lower titer of antibody in the IGIV because of the large quantity of antibody administered and the complete availability of the IGIV. They suggest that IGIV could be used pro-

phylactically instead of intramuscular VZIG. It also is preferable in individuals with thrombocytopenia, in whom intramuscular injections are contraindicated.

In sum, VZIG (or IGIV) is useful in modifying the severity of varicella-zoster infections in immunocompromised hosts but will not reliably prevent its occurrence. ZIP¹⁸⁵ used at a dose of 10 ml/kg is equally efficacious in preventing infection, but it is not commercially available and risks transmitting hepatitis and other viral diseases.

Recommendations

For normal children exposed to varicella, standard HISG can be expected to modify the disease and VZIG to prevent it.¹⁷⁹ However, since varicella is a mild disease in most children, prophylaxis is usually not indicated. In high-risk immunocompromised children and in normal and high-risk adults, the decision to administer VZIG or HISG is based on the likelihood of susceptibility, the nature of the exposure (Table 7), and the risk of developing varicella (Table 8).¹⁸⁶ VZIG is expensive (at least \$75 for 125 IU; at least \$375 for an adult over 40 kg), and the modified infection that

TABLE 7.
Exposure Criteria* for Which VZIG is
Indicated†

1. One of the following types of exposure to persons with chickenpox or zoster:
 - a. Continuous household contact.
 - b. Playmate contact (generally >1 hour of play indoors).
 - c. Hospital contact (in same two- to four-bed room or adjacent beds in a large ward or prolonged face-to-face contact with an infectious staff member or patient).
 - d. Newborn contact (newborn of mother who had onset of chickenpox 5 days or less before delivery or within 48 hours after delivery).

AND

2. Time elapsed after exposure is such that VZIG can be administered within 96 hours but preferably sooner.

*From Centers for Disease Control: Varicella-zoster immune globulin for the prevention of chickenpox: Recommendations of the Immunization Practices Advisory Committee. *Ann Intern Med* 1984; 100:859-865.

†Patients should meet both criteria.

TABLE 8.
Persons for Whom VZIG Is Indicated*

Susceptible to varicella-zoster
Significant exposure
Less than 15 years of age, with administration to immunocompromised adolescents and adults and to other older patients on an individual basis
One of the following underlying illnesses or conditions:
Leukemia or lymphoma
Congenital or acquired immunodeficiency
Immunosuppressive treatment
Newborn of mother who had onset of chickenpox within 5 days before delivery or within 48 hours after delivery
Premature infant (≥ 28 weeks gestation) whose mother lacks a prior history of chickenpox
Premature infants (< 28 weeks gestation or $\leq 1,000$ gm) regardless of maternal history

*From Centers for Disease Control: Varicella-zoster immune globulin for the prevention of chickenpox: Recommendations of the Immunization Practices Advisory Committee. *Ann Intern Med* 1984; 100:859-865.

ensues may not lead to lifelong immunity and may not decrease the risk of developing zoster later in life.

Determination of Susceptibility.—Determination of susceptibility is usually based on historic information elicited by an experienced interviewer. Varicella-zoster virus antibody tests to determine varicella susceptibility are not widely available and are often unreliable. Complement fixation, fluorescent antibody, ELISA, and neutralizing antibody tests are used, but they may be negative despite immunity and, when positive, may not indicate immunity, particularly in neonates and immunocompromised subjects. Table 9 summarizes the determination of susceptibility.

With the exception of bone marrow transplant recipients, healthy and immunocompromised individuals who by history have had prior varicella infection are considered immune. Healthy adults and children 15 years of age and older with a negative or uncertain history are generally considered immune; however, those who are immunocompromised are considered susceptible. Approximately 85% to 95% of adults and children 15 years of age and older with a negative or uncertain history of prior varicella are immune, particularly those who are older siblings in large families and those whose children have had varicella. Children who are younger than 15 years of age and do not have a history of varicella are considered susceptible unless proven otherwise using a sensitive serologic assay; how-

TABLE 9.
Determination of Susceptibility to Varicella in Some Selected Situations*†

Group	Immune Status	Carefully Obtained Prior History of Varicella	Detectable Varicella Antibody by ■ Reliable Test	Susceptibility Status
Children (<15 years)	Immunocompromised	Yes	→ Unnecessary to perform	→ Immune
		No or unknown	→ ‡	→ Susceptible
Adolescents and adults (≥15 years)	Normal	Yes	→ Unnecessary to perform	→ Immune
		No or unknown	→ Not performed	→ Generally consider immune‡
			→ Yes	→ Immune
			→ No	→ Susceptible
Immunocompromised	Immunocompromised	Yes	→ Unnecessary to perform	→ Immune
		No or unknown	→ ‡	→ Consider susceptible§

*From Centers for Disease Control. Varicella-zoster immune globulin for the prevention of chickenpox: Recommendations of the Immunization Practices Advisory Committee. *Ann Intern Med* 1984; 100:859-865.

†This table provides general guidelines for determining susceptibility in frequently encountered situations. Not all potential scenarios are considered. In all situations, individual judgment should also be used. See text for details.

‡Some immunocompromised persons with detectable antibody before VZIG administration, presumably passively transferred by recent transfusions, have developed clinical varicella. Until further evaluation of serologic tests in the immunocompromised has been completed, one may have to rely on a carefully obtained clinical history by an experienced interviewer to determine susceptibility (i.e., the absence of a history of clinical varicella).

§More than 85% and probably more than 95% of such persons are immune.

ever, results of these assays must be interpreted cautiously in immunocompromised children. Children or adults who have received bone marrow transplants should be considered susceptible to varicella regardless of prior history of the disease in the donor or recipient. Bone marrow transplant recipients who develop varicella or herpes zoster following transplantation can subsequently be considered immune.

Normal Children.—As noted, prophylaxis for most normal children is not indicated. However, special circumstances (e.g., future travel, surgery, risk of exposure of an immunocompromised sibling) may require its use; the dose is identical to that for immunocompromised children.

Immunocompromised Children.—The principal use of VZIG is following exposure to zoster or chickenpox (see Table 8) of susceptible immunocompromised children. This includes children with primary immunodeficiency, with neoplastic diseases, and those receiving immunosuppressive therapy including corticosteroids. VZIG may only modify varicella rather than prevent it. Although children who have neoplastic disease and children receiving immunosuppressive therapy and who have a definitive history of varicella are considered immune, patients with primary antibody or cellular immunodeficiency or children receiving bone marrow transplants should be considered to be susceptible regardless of prior history.

Newborns.—VZIG is recommended for a newborn whose mother develops varicella within 5 days before or 48 hours after delivery, regardless of whether the mother received VZIG. Varicella infection is life threatening in newborns, and VZIG may be expected to modify but not prevent the disease. VZIG is probably unnecessary for term newborns whose mothers develop varicella more than 5 days before delivery, inasmuch as they will receive some transplacental immunity and will be protected from severe disease.

Postnatal exposure to varicella infection does not represent a threat to normal infants, particularly if the mother has had varicella, since transplacental antibodies will modify or protect the infant. In all exposed infants less than 28 weeks' gestation or weighing less than 1,000 gm, VZIG is recommended because transplacental passage of IgG is significantly lessened and the premature's cellular immune system is particularly immature. In larger exposed prematures, VZIG is recommended for those whose mothers have not had varicella.

Immunocompromised Adults.—Exposed immunocompromised adults who are believed susceptible should receive VZIG. Many (most) are probably immune, but in this high-risk situation, prophylaxis is justified.

Normal Adults.—Adults are more susceptible to severe varicella than are children; the death rate from varicella in adults is 50 per 100,000 compared with 2 per 100,000 in children, and the complication rate is increased 9- to 25-fold. Accordingly, some exposed adults, depending on their health and the need to avoid or modify varicella, are candidates for

VZIG. Although the cost of VZIG is substantial, under certain circumstances, the benefits clearly outweigh this expense.

Pregnant Women.—Exposed pregnant women should be considered the same as other exposed adults. There is no evidence that VZIG given during pregnancy will prevent intrauterine, congenital, or neonatal varicella. It is unlikely that VZIG given to a pregnant woman with varicella within 5 days of delivery will result in sufficient transplacental passage to modify or prevent varicella in her infant. Accordingly, the infant should be given VZIG regardless of whether the mother has received VZIG prenatally.

Hospital Personnel and Exposures.—Recommendations for exposed hospital personnel and ward management of exposure problems are available.¹⁸⁶

Dosage.—VZIG should be given as soon as possible after exposure but probably is effective if given within 4 or 5 days after exposure. VZIG is not of value in the treatment of varicella or zoster. VZIG probably confers protection for 3 weeks. Antibody determinations before and 2 months after administration may help to determine the immune status of the individual and whether subclinical or modified infection has resulted.

VZIG is supplied in vials of 125 units (about 1.25 ml). The recommended dose is 125 units/10 kg, up to a maximum of 625 units (5 vials). The minimum dose is 125 units. The necessity for higher doses in adults weighing more than 50 kg is not established. The cost is at least \$75 per vial. The product is prepared by the Massachusetts Public Health Biologic Laboratories and is distributed through pharmacies and regional distribution centers of the American Red Cross.¹⁸⁷ If VZIG is unobtainable or unaffordable, standard HISG, or IGIV may be used. The dose of HISG (IGIM) is 0.6 to 1.2 ml/kg and the dose of IGIV is 300 mg/kg.

Use of Animal Serums and Antitoxins

No discussion of passive immunity is complete without noting the continued availability of several animal sera for therapeutic use (Table 10). In certain situations there is no alternative to the use of sera (e.g., diphtheria antitoxin in diphtheria, snake bite antivenom following bites) since special human immune globulins are not available. In other situations (e.g., tetanus, rabies exposure) both animal antisera and special human immune globulins are available; the latter are preferred.

Animal sera are also used as immunosuppressive agents in the form of equine antithymocyte globulin or murine monoclonal anti-T cell antibody. Another animal antibody (Digoxin immune FAb, Digibind) is available for the treatment of digoxin toxicity. It is an ovine (sheep) anti-digoxin antibody that has been partially digested with papain. It binds to digoxin and the complex is excreted rapidly by the kidney.

TABLE 10.
Animal Antiserums and Antitoxins Available

Product	Abbreviation	Use
Tetanus antitoxin	TAT	Prevention or treatment (when TIG unavailable)
Diphtheria antitoxin		Prevention or treatment
Rabies immune serum		Prevention (when RIG unavailable)
Botulism antiserum		Treatment of botulism
Black widow spider antivenin	ATGAM	Treatment of spider bite
Crotalidae antivenin		Treatment of snake bite
Antithymocyte globulin		Immunosuppression
Anti T3 monoclonal Ab		Immunosuppression
Digoxin antibody	Orthoclone OKT3	Treatment of digoxin toxicity
	Digibind	

Precautions

Animal sera are derived from immunized animals, usually horses (equine) or rabbits. Because these serums are foreign proteins, there is a significant risk to their use. Thus, they should be administered only when specifically indicated, after sensitivity tests, and by a physician prepared to treat a hypersensitivity reaction.

A careful history must be taken before an animal serum is injected. Patients with a history of asthma, allergic rhinitis, or other allergic symptoms on exposure to horses or rabbits may be dangerously sensitive to the corresponding serum and should receive it only with the utmost caution. A scratch test or eye test, followed by an intradermal skin test, always should be performed before an injection of animal serum, whether or not the patient has had the serum previously. These tests indicate the probability of sensitivity. However, a negative test is not an absolute guarantee of absence of sensitivity. Therefore, either a specific history of allergy or a positive skin or eye test with horse serum is sufficient reason for special caution. A positive history of sensitivity to horse dander is an indication of the need for extreme caution.

If the history and sensitivity tests are negative, the indicated dose of serum may be given intramuscularly. Intravenous injection may be indicated if a high concentration of circulating antibody is required rapidly, as in severe tetanus or diphtheria. Schedules for administration of horse serum intravenously are available.¹⁸⁸

Hypersensitivity Reactions

Hypersensitivity reactions to animal serum may be of four general types: (1) anaphylactic reactions, consisting of urticaria, dyspnea, cyanosis, shock, and unconsciousness occurring seconds to minutes after an injection; (2) acute febrile reactions, consisting of moderate or severe hyperpyrexia within 2 hours after an injection; (3) serum sickness reactions, consisting of urticaria, arthritis, adenopathy, and fever occurring hours to days after an injection, depending on the dose and the presence or degree of prior sensitization (serum sickness occurs within hours or a few days after the second injection and within 7 to 12 days after the first injection); and (4) delayed reactions of varying nature, including peripheral neuritis (serum neuritis).

Use of Human Intravenous Immune Globulin

Pharmacology of Intravenous Immune Globulins

IGIVs for therapeutic use have been available since 1981 and have emerged as important therapeutic agents. IGIVs are prepared by eliminat-

ing high-molecular-weight complexes and their resultant anticomplementary activity. Methods to accomplish this have utilized (1) physical removal of aggregates by ultracentrifugation or gel filtration, (2) treatment with proteolytic enzymes, (3) treatment with chemicals that reduce sulphhydryl bonds, followed by alkylation of the free-SH bonds, and (4) incubation at low pH. Physical removal of aggregates is impractical. Four IGIVs have been licensed in the United States (Table 11) and others are in use elsewhere.

The first IGIV licensed in the USA was a reduced (dithiothreitol) and alkylated (iodoacetamide) product formulated as a 5% solution in 10% maltose (Gamimune, Cutter Laboratories).^{189*} The second IGIV licensed (1984) was an acidified, pepsin-treated powder reconstituted to a 3% or 6% solution in 5% or 10% sucrose (Sandoglobulin, Sandoz Laboratories). The third, Gammagard, a 5% solution in glycine, is prepared by ion-exchange chromatography, and the fourth, Gamimune-N, a 5% solution in maltose, is acidified at pH 4.25. All of these approach, but do not achieve, the characterization of an ideal IGIV (Table 12). For most patients and illnesses, these products are equally efficacious. For patients with anti-IgA antibodies, Gammagard is the preparation of choice, since it has the lowest level of IgA.¹⁸⁷

These preparations have acceptable serum half-lives (18 to 25 days), minimal anticomplementary activity, good antibody content, and HBsAg and HIV antibody negativity. A few cases of non-A, non-B hepatitis with IGIV have been recorded.^{190, 191} Donors are now screened for non-A, non-B hepatitis by measuring their alanine aminotransferase levels.¹⁹²

These IGIV preparations are equally effective in preventing serious infections in antibody-deficient subjects as are equivalent doses of HISG given by intramuscular injections.¹⁸⁸ The incidence of side effects (other than local pain) such as nausea, vomiting, flushing, chest tightness, coughing, and chills is significantly increased threefold or fourfold but usually not life threatening. Anti-IgA antibodies of the IgE class have been responsible for one life-threatening reaction.¹³ Most patients who do not have such reactions prefer IGIV. The incidence of side reactions to IGIV can be reduced by pretreatment with aspirin, diphenhydramate, or corticosteroids.¹⁹³⁻¹⁹⁵

For adults requiring large volumes of intramuscular HISG injections, IGIV therapy is much better tolerated. In patients with thrombocytopenia (e.g., Wiskott-Aldrich syndrome, patients undergoing bone marrow ablation for transplantation), IGIV is the agent of choice. Another advantage of IGIV is the large quantities of IgG that can be administered; indeed IgG levels can be normalized (IgG > 600 mg/dl) with repeated daily infusions.

IGIV can be used in illnesses other than antibody deficiency—a fact that was not recognized during its development. The benefit in some illnesses was unexpected, as in Kawasaki's disease and idiopathic thrombocytopenia.

*No longer available.

TABLE 11.
Licensed Intravenous Immunoglobulins in the United States*

Name	Isolation Method	Product Form	Comments
Gamimune (1981) ^{†‡}	Reduced and alkylated	5% in maltose; 50-ml vial (2.5 gm)	Low in IgG3 and IgG4 Lowest in IgA
Sandoglobulin (1984) [§]	Acid and pepsin	3% or 6% in sucrose 1-, 3-, or 6-gm powder	
Gammagard (1986) [¶]	Chromatography	5% in glycine; 2.5 or 5 gm powder	
Gamimune-N (1986) [†]	Acid (pH 4.25)	5% in maltose; 10-, 50-, or 100-ml vials	

*From Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367. Used by permission.

[†]Cutter Laboratories, Berkeley, California.

[‡]No longer available.

[§]Sandoz Inc., Pharmaceutical Division, East Hanover, New Jersey.

^{||}Lyophilized; reconstitute before use with diluent provided.

[¶]Travenol Laboratories, Inc., Deerfield, Illinois.

TABLE 12.
Properties of an Ideal Intravenous Immunoglobulin*

Wide spectrum of antibodies (donor pool greater than 1,000 donors)
 Long serum half-life (greater than 20 days)
 Contains mostly monomeric IgG (greater than 90%); all IgG subclasses
 Contains no pyrogens, aggregates, other vasoactive substances, not anti-complementary
 Minimal side effects
 Minimal IgA, other serum proteins
 Sterile (no hepatitis B virus, human immunodeficiency virus, other pathogens)
 Stable, readily soluble (if a powder)
 Inexpensive

*From Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367. Used by permission.

nia purpura (ITP); the benefit in certain infections was less surprising because large quantities of specific antibody are given.

IGIV in Antibody Deficiencies

IGIV can be used for the primary and secondary antibody deficiencies that were shown in Table 4. The advantages and disadvantages of IGIV over HISG for the treatment of antibody deficiency are shown in Table 13. Used at the same 100 mg/kg/month, IGIV and HISG provide similar therapeutic effects.¹⁸⁹

Administration of IGIV usually requires 1 to 3 hours. If a reaction is anticipated, premedication is given 1 hour before the infusion is started. The infusion is begun at a slow rate (e.g., 0.5 ml/minute). If this is tolerated, the infusion can be increased to 1 to 3 ml/minute. IGIV is generally well tolerated and efficacious in the treatment of antibody deficiency. Large doses, even given daily, can be given with impunity. Because drug toxicity does not limit dosage, other end points must be used as guidelines to the optimal dose.

Dosage

The serum IgG level increases in a dose-dependent fashion immediately after an IGIV infusion. In general each 100 mg/kg of IGIV infused increases the IgG level 200 mg/dl above the preinfusion IgG level. The IgG level drops off rapidly for 1 to 3 days immediately after an infusion due to

TABLE 13.
Advantages and Disadvantages of
Intravenous Immunoglobulin
Compared With Intramuscular
Immunoglobulin*

Advantages of intravenous immunoglobulin

- Less painful
- No pooling within tissues
- No sterile abscesses
- No mercury exposure
- No volume limitation
- Rapid attainment of blood levels
- Less frequent injections
- High levels feasible
- Half-life studies feasible
- Use in other disorders
- Daily treatment feasible

Disadvantages of immunoglobulin

- Expensive
- Venous access necessary
- Longer time of administration
- More frequent side effects (5% to 15%)
- More severe side effects
- Hepatitis (a few cases)

*From Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367. Used by permission.

equilibration throughout the distribution compartment.¹⁹⁶ Thereafter, the IgG levels fall exponentially with a half-life of about 20 days. There have been several methods proposed to individualize dosage using half-life determinations.¹⁹⁷ However, the administered dose may not achieve the expected serum levels. Perhaps the half-life is shortened by the higher serum level or there is excessive loss within the extravascular space in the early equilibration phase. Among different persons, the IgG half-life ranges from 11 to 44 days (mean, 25.4 days).¹⁹⁸ For patients with a short IgG half-life, more frequent infusions of a smaller IGIV dose can be used to maintain IgG levels in the normal range. In most patients, normalization of serum IgG levels can be achieved by increasing the dose or frequency of administration. However, serum levels do not necessarily correlate with clinical benefit.

Prevention of infection is the ultimate goal in IGIV therapy in patients

with antibody deficiency. HISG doses of 100 mg/kg/month given intramuscularly are effective in preventing serious infections when compared with no treatment.¹⁰ Although higher doses do not reliably provide additional clinical benefit, two studies have indicated fewer infections with a higher dosage. Nolte et al.¹⁹⁹ compared 150 mg/kg/month of IGIV with 100 mg/kg/month of HISG given intramuscularly. Cunningham-Rundles et al.²⁰⁰ compared 300 mg/kg/month of IGIV with 150 mg/kg/month of HISG given intramuscularly. Both studies showed that patients given a higher IGIV dose had significantly fewer acute infections. The route of administration (IV vs. IM) may have influenced the outcome.

In contrast to these studies, a recent multicenter study comparing 100 mg/kg/month of IGIV with 400 mg/kg/month of IGIV showed no reduction in infections even though high-dose patients had normal IgG levels.¹⁹³ The inability to reduce the number of infections by high-dose IGIV may reflect an inability to deliver antibody to the mucous membranes. In addition, patients with chronic infection (e.g., bronchiectasis) may never be infection-free, despite massive infusions of IGIV. Another study using an IGIV dose of 500 mg/kg/month provides some evidence that a high dose may reduce the number of infections when compared with IGIV at a dose of 150 mg/kg/month.²⁰¹

Our practice is to initiate IGIV therapy at doses of 150 to 200 mg/kg at 3- or 4-week intervals; we try to keep trough IgG levels over 400 mg/dl. If infections persist after 6 months on this regimen (or on intramuscular injections), we increase the IGIV dose to 400 to 500 mg/kg to maintain the IgG level over 600 mg/dl. Some but not all patients do better on the higher dosage.

Self-Administration of IGIV

Another major consideration in determining the dose of IGIV is economics. A 75-kg man receiving 400 mg/kg/month of IGIV will spend about \$1,200 per month for IGIV, excluding administration costs and physician fees. Most patients now receive their monthly IGIV as a 3- to 4-hour outpatient visit. Ashida and Saxon²⁰² have developed a program for the home self-administration of IGIV for patients with antibody deficiency based on the finding that hemophiliacs can self-administer factor VIII.

Seven patients with antibody deficiency, selected for their ability to learn the procedure and for the possession of readily accessible veins, were begun on this program. The material needed for the infusions is available at local pharmacies (Table 14). The patients use a small-size butterfly needle inserted into a hand or forearm vein. An infusion pump, such as Auto-Syringe model AS2F (Auto Syringe, Hooksett, New Hampshire), permits the patient to regulate the rate of infusion. Because of the potential for anaphylactic reactions, each patient must have an Ana-Kit (Hollister-Stier, Spokane, Washington) or EpiPen (Center Laboratories, Port Washington, New York) available during the infusion.

During a 3-year period, this method has proven to be safe, effective,

TABLE 14.
Materials Needed for Home Intravenous
Immunoglobulin Infusions Using Pump*

For venipuncture
Tourniquet
23-gauge 0.75-inch butterfly needle with 12-inch tubing
Alcohol swabs
Gauze
Bandaid
12-inch syringe for 5% dextrose in water to flush intravenous line
5% dextrose in water, sterile
Tape
For infusion
35-ml syringes to fill with intravenous immunoglobulin and load in AutoSyringe† pump
18-gauge needles to use with 35-ml syringes
33-inch intravenous tubing to connect 35-ml syringe and 23-gauge butterfly needle
Infusion pump (AutoSyringe†)
Intravenous immunoglobulin preparation
Other
Ana-Kit‡ or EpiPen§

*From Ashida ER, Saxon A: Home intravenous immunoglobulin by self administration. *J Clin Immunol* 1986; 6:306–309. Used by permission.

†AutoSyringe, Auto Syringe, Hooksett, New Hampshire.

‡Ana-Kit, Hollister-Stier, Spokane, Washington.

§EpiPen, Center Laboratories, Port Washington, New York.

and cost efficient with good patient acceptance; there have been no adverse reactions to the IGIV. Home infusion has been equivalent to hospital-based infusion in terms of number of minor and major infections, days missed from work, amount of antibiotics used, and IgG levels achieved. Patients can administer the IGIV at their convenience without missing work or incurring hospitalization expenses. Thus, self-administration of IGIV helps to reduce the enormous costs of these lifelong diseases.

IGIV in Infections

The use of IGIV in the therapy of certain viral and bacterial infections is under extensive study. In experimental group B streptococcal infection in newborn rats, IGIV plus antibiotics are superior to antibiotics alone in clearing the blood stream of organisms.²⁰³ Encouraging preliminary results

are also available for experimental infections with *Klebsiella*, *Pseudomonas*, and *E. coli*. Routine use of IGIV in human bacterial infection is premature; a few clinical reports suggest that IGIV may be contraindicated in the therapy of severe infection, possibly because of reticuloendothelial blockage.²⁰⁴⁻²⁰⁶

Neonatal Infections

IGIV has been used in neonatal infections with suggestive therapeutic benefits.²⁰⁷ IGIV may also be of value in treating overwhelming infections due to *Hemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitis*.²⁰⁸⁻²¹¹ A new immunoglobulin, termed bacterial polysaccharide immunoglobulin (BPIG), derived from volunteers immunized with capsular polysaccharides of *H. influenzae* type b, pneumococci, and meningococci²¹⁴ is effective in preventing bacteremia and meningitis caused by *H. influenzae* type b in infant rats.²¹¹ It also provides significant protection against *H. influenzae* type b bacteremia and meningitis in Apache infants.²¹² Use of this hyperimmune globulin may provide an alternative approach to active immunization for prevention of disease due to *H. influenzae* type b in certain high-risk populations, such as Alaskan Eskimos and native Americans.

Cytomegalovirus Infections

There have been several trials of IGIV in bone marrow transplant patients at risk for symptomatic cytomegalovirus (CMV) infection and pneumonia. Winston²¹³ has summarized the results of six controlled trials (over 300 patients) of CMV immune plasma, CMV hyperimmune globulin, or polyvalent IGIV containing CMV antibody (Gamimune).

The latter IGIV was given at high doses (20 ml/kg every week for 4 months) and was well tolerated.²¹⁴ The incidence of CMV infection was 35% in the recipients of immune plasma or globulin versus 51% in control subjects, and CMV pneumonia was reduced from 23% to 8%. These results suggest that immune globulin or plasma, although not necessarily preventing CMV infection, modifies the severity of infection and prevents CMV interstitial pneumonia in bone marrow transplants.

Similar results have been obtained in CMV interstitial pneumonia in bone marrow and renal transplant recipients. In a multicenter study of prophylactic intravenous CMV hyperimmune globulin in renal transplants,²¹⁵ the incidence of symptomatic CMV infection (fever, leukopenia, thrombocytopenia, hepatitis, or pneumonia) was reduced from 64% (14 in 22 control subjects) to 20% (three in 15 globulin recipients). CMV viremia and other opportunistic infections also occurred less frequently in the globulin recipients.

In addition to prophylaxis, IGIV has been used for the treatment of established CMV infections. Condie et al.²¹⁶ reported that 71% of 52 renal transplant patients with life-threatening CMV infection improved after treatment with IGIV, and a favorable response correlated with the postinfusion

CMV antibody titer. Similarly, five of seven renal transplant patients treated by Nicholls et al.²¹⁷ with a CMV hyperimmune IGIV for severe CMV infection showed significant clinical improvement. Six of nine bone marrow transplant patients with CMV pneumonia treated with a CMV immunoglobulin in London survived.²¹⁸ By contrast, only two of ten patients at another center survived CMV pneumonia after treatment with CMV immunoglobulin.²¹⁹ Thus, IGIV is not of proven value in the therapy of established CMV infection.

Chronic Meningoencephalitis

Chronic meningoencephalitis due to echovirus characteristically occurs in patients with X-linked agammaglobulinemia. Treatment with high doses of IGIV modifies the severity of infection and improves survival. Winston's review of published reports²¹³ indicated that eight of 11 patients treated with IGIV survived compared with only two of ten patients given intramuscular HISG or immune plasma and none of four patients who received no treatment.^{220–223} Two patients who received intraventricular immunoglobulin as well as IGIV experienced resolution of cerebrospinal fluid (CSF) pleocytosis with eradication of the echovirus from the CSF.²²⁴ However, other patients, including two given intraventricular immunoglobulin,^{223, 225} have a chronic fluctuating course with persistent CSF pleocytosis and positive CSF viral culture.

Varicella-Zoster

The varicella-zoster antibody titers achieved after administration of Gamimune (4 to 6 ml/kg) are equivalent to those achieved after VZIG.¹⁸⁴ Administration of this IGIV may be useful prophylactically in patients with known exposure to varicella-zoster when VZIG is not available or cannot be given because of thrombocytopenia. VZIG or IGIV is not of value in the treatment of established varicella-zoster infections.⁸³

Other Viral Infections

In chronic Epstein-Barr disease, a preliminary uncontrolled study reported symptomatic improvement following IGIV therapy in eight of 12 patients.²²⁶ Sullivan²²⁷ suggests the use of IGIV in unexposed infants and children with the X-linked lymphoproliferative syndrome to prevent Epstein-Barr infection; these boys develop severe and sometimes fatal complications from this virus infection.

IGIV preparations also contain antibodies to several common respiratory tract viral pathogens including respiratory syncytial virus, adenovirus, influenza, and parainfluenza.^{228–231} In animal models, the administration of IGIV modifies the severity of respiratory syncytial virus infection^{232, 233} with significant reduction of viral shedding from the lungs and nose. A double-blind, placebo-controlled trial of high dose IGIV for the treatment of hospitalized children with respiratory syncytial virus lower respiratory tract infection is underway.²³⁴

There are no controlled trials on the efficacy of IGIV for other viral lower respiratory tract infections. Adenovirus pneumonia has occurred in immunodeficient children lacking neutralizing antibody to adenovirus, and one child recovered after receiving intramuscular immunoglobulin.²³⁵ In contrast, IGIV failed to eradicate parainfluenza pneumonia in a child with severe combined immunodeficiency.²³⁶

A unique use of IGIV preparations is administering them orally to immunodeficient patients with viral gastroenteritis. Pharmacokinetic studies indicate that immunoglobulin possessing antirotavirus activity survives gastrointestinal passage in an immunologically active form and decreases the amount of uncomplexed rotavirus antigen in the gastrointestinal tract by formation of rotavirus-specific immune complexes.²³⁷ Prophylactic oral immunoglobulin protected low-birth-weight infants from rotavirus diarrhea in a nursery where rotavirus was endemic.²³⁸

Pediatric AIDS

Calvelli and Rubinstein²³⁹ have used IGIV to prevent infections in children with pediatric acquired immune deficiency (PAIDS). These patients have a functional antibody defect and suffer from repeated bacterial and viral respiratory infections (as well as several opportunistic infections). They seem better when kept on monthly or every 3 weeks IGIV at doses of 200 to 400 mg/kg/month. Some PAIDS patients have improved their *in vitro* T cell immunity. In one study, 12 of 14 infants treated with IGIV survived whereas 14 of 27 comparable patients who did not receive IGIV survived.²³⁹ The use of IGIV containing antibody to HIV virus in PAIDS is under study to determine if this provides better clinical results than IGIV without detectable HIV antibodies.

Of note, there is no risk of transmitting HIV virus or other viruses via HISG or IGIV.^{240, 241} The alcohol fractionation procedure removes and inactivates these viruses.²⁴² Since HISG or IGIV collected before 1985 were derived from donor pools that included some HIV antibody positive individuals, these products contained HIV antibodies and recipients could transiently become HIV antibody positive. Present lots of IGIV have no HIV antibodies. Certain lots of HBIG and other special immune globulins may still contain significant HIV antibody titers.

IGIV in Immunoregulatory Disorders

IGIV in Idiopathic Thrombocytopenic Purpura

The therapeutic benefit of IGIV in ITP was first reported by Imbach et al.^{243, 244} who noted that the thrombocytopenia in several agammaglobulinemic and Wiskott-Aldrich syndrome children improved following IGIV therapy. When this therapy was extended to other pediatric patients with

thrombocytopenia, they noted that ITP patients had higher platelet counts and diminished bleeding following IGIV administration.

Since then, high-dose IGIV (at least 400 mg/kg/day for 3 to 5 days) has been used extensively in both acute and chronic ITP in children and adults.^{244, 245} Several different commercial IGIVs have been used successfully;²⁴⁶ they are probably therapeutically equivalent.

In children with acute ITP, the risk of life-threatening hemorrhage early in the disease is the major concern. Therefore, attention has focused on how rapidly patients treated with IGIV achieve a sustained rise in platelets. Bussel et al.²⁴⁷ treated 29 patients with IGIV (1 gm/kg/day for 3 days); all experienced a marked increase in the platelet count within 24 hours. Imbach et al.²⁴⁸ compared 47 children with acute ITP treated with IGIV (400 mg/kg/day for 5 days) to 47 children treated with corticosteroids. In most patients (62%), the response to either therapy was equally rapid, but in the remainder (38%), patients treated with IGIV achieved remission sooner. The incidence of progression to chronic ITP was the same in both groups (about 20%).

Because the risk of severe hemorrhage is greatest with extremely low platelet counts, IGIV has been recommended in acute thrombocytopenic purpura when the platelet count falls below 10,000/ μ l.²⁴⁹ One strategy has been to initially treat these children with corticosteroids; if there is not a prompt increase in the platelet count, IGIV can be added to bring about a more rapid remission.

IGIV has also been used in chronic ITP when conventional therapy has been relatively ineffective; children with this disease may respond better than adults. Bussel et al.²⁵⁰ gave IGIV to 12 children with chronic ITP who were also receiving corticosteroids and were being considered for splenectomy. IGIV was given at doses of 400 mg/kg/day for 5 days followed by single infusions of 400 mg/kg/day, first weekly and then monthly as needed to maintain the platelet count of 24,000/ μ l to an average peak posttreatment count of 266,000/ μ l. At follow-up, at least 4 months after maintenance therapy was stopped, one of 12 patients had achieved complete remission and four of 12 had maintained platelet counts more than 40,000/ μ l. Thus, IGIV may permit the postponement or avoidance of splenectomy in some children. It may also permit the discontinuation of immunosuppressive medication that is causing undesirable side effects.

Mori et al.²⁵¹ gave IGIV (400 mg/kg/day for 5 days) to 25 children with chronic ITP without subsequent infusions; the results, however, were less dramatic. Twenty responded with significantly increased platelet counts but in only three did platelet counts remain over 100,000/ μ l for at least 4 months. In the remaining 17, the platelet count returned to pretreatment values within 1 to 4 weeks. Neither study had treated controls so it is difficult to determine whether IGIV truly deferred the need for splenectomy.

Several mechanisms of action for the beneficial effects of IGIV in thrombocytopenia have been proposed. IGIV delays the clearance of antibody-coated autologous erythrocytes; this parallels an increase in the platelet count suggesting a competitive blockade of reticuloendothelial Fc receptors.^{244, 252}

Platelet-associated immune globulin decreases after successful IGIV therapy,^{253, 254} suggesting that IGIV decreases antiplatelet antibody production. Monomeric IgG inhibits pokeweed mitogen induced B-cell differentiation, presumably by an antigen-nonspecific effect on antibody production.²⁵⁵ Alternatively, the presence of anti-idiotypic antibodies in IGIV may successfully downregulate antibody production.²⁵² IGIV may also interfere with the interaction of platelets with immune complexes, antiplatelet antibodies, or both.^{256, 257}

IGIV in Other Immune Thrombocytopenias

The success of IGIV in ITP has prompted its use in several other immunologically mediated thrombocytopenias. IGIV given to patients with leukemia who are sensitized and refractory to random donor platelets will prolong the survival of exogenous platelets during episodes of active bleeding.^{260, 261}

Neonatal thrombocytopenic purpura secondary to maternal ITP and resulting transplacental maternal antibody has been successfully reversed with IGIV;²⁶² IGIV given prenatally to mothers with ITP has prevented neonatal thrombocytopenic purpura in some,^{263, 264} but not all, cases²⁶⁵ despite attainment of normal maternal platelet counts. Isoimmune thrombocytopenia in the newborn period due to maternal isoimmunization has also been treated successfully with IGIV. Massey et al.²⁶⁶ reports prompt reversal of thrombocytopenia in three such infants.

Posttransfusion purpura has also responded to IGIV,^{260, 267} as has thrombotic thrombocytopenic purpura,²⁶¹ and gold-induced purpura.²⁶⁸ IGIV has been used successfully in postinfectious thrombocytopenia—that associated with varicella-zoster infection²⁶⁹ or HIV infection.²⁷⁰

IGIV In Neutropenia and Autoimmune Hemolytic Anemia

IGIV has also been used in the treatment of autoimmune neutropenia in a steroid-dependent adult²⁷¹ and in three infants.^{272, 273} A remission was induced in one infant and transiently increased neutrophil counts in the other three patients, with resolution of infection. IGIV may therefore provide a useful alternative to immunosuppressive agents, particularly during episodes of infection.

IGIV has been used in autoimmune hemolytic anemia with both success and failure^{274, 275} unrelated to the age of the patient or the IGIV dose. Because of its safety, an early trial of IGIV (400 mg/kg/day for 5 days) is probably warranted, particularly if the response to steroids is unsatisfactory.

IGIV in Kawasaki's Disease

IGIV is of benefit in the therapy of Kawasaki's disease, an acute self-limited febrile illness of children whose major complication is coronary aneurysm.²⁷⁶⁻²⁷⁸ The pathologic findings suggest a vasculitis similar to infantile polyarteritis nodosa.²⁷⁷ Coronary aneurysms develop in about 20% of patients and the associated coronary thrombosis accounts for a 1% to 2% mortality.^{277, 278} Conventional therapy consists of high-dose aspirin for its antiinflammatory and antithrombotic effects, but it seems to have little effect on the incidence of coronary aneurysm.²⁷⁹ Because circulating immune complexes have been implicated in the pathogenesis of Kawasaki's disease and IGIV is known to modulate Fc receptor function in thrombocytopenic purpura, IGIV has been used.

An initial study from Japan compared IGIV (400 mg/kg/day for 5 days) plus aspirin (30 mg/kg/day) to aspirin therapy alone.²⁸⁰ After 60 days of therapy, three of 40 (8%) of the IGIV-aspirin patients had persistent coronary aneurysms by echocardiography vs. 14 of 45 (30%) in the control group. There was no difference in duration or height of fever, platelet count, or acute-phase reactants. Because the echocardiographs were not read blind, the validity of the study is questioned.

A recent trial from the USA compared 84 patients treated with IGIV (400 mg/kg/day for 4 days) plus aspirin (100 mg/kg/day for 2 weeks followed by 3 to 5 mg/kg/day) with 84 control patients treated with aspirin alone.²⁸¹ Echocardiography was done at 2 and 7 weeks after treatment and interpreted blind. At 7 weeks, three of 79 (4%) of treated patients had aneurysms versus 14 of 79 (18%) of control patients. In the treatment group, fever resolved more quickly and white blood cells and neutrophil levels were significantly lower, whereas platelet counts were comparable. Similar results were reported from Japan by Nagashima et al.,²⁸² using 400/mg/kg/day for 3 days, coronary abnormalities occurred in 25 of 65 (37%) aspirin-treated patients vs. 11 of 69 (16%) of the IGIV treated group. Long-term follow-up will be necessary to determine the ultimate effect of IGIV on morbidity and mortality.

As in ITP, the mechanism of action of IGIV in Kawasaki's disease is not known. Possible Fc receptor blockade or modulation of function, suppression of antibody synthesis, or administration of specific antibody to neutralize an as yet unidentified retrovirus of Kawasaki's disease have been suggested. Lueng et al.²⁸³ noted that IGIV treatment of Kawasaki's disease diminished the levels of activated T helper cells and decreased the secretion of T cell derived B cell growth factors.

IGIV In Other Immunoregulatory Disorders

Case reports have detailed the successful use of IGIV in several other autoimmune disorders.

Myasthenia gravis is associated with antiacetylcholine receptor antibody-

ies that can be passed transplacentally to neonates. Myasthenia is also associated with other autoimmune antibodies and autoimmune disorders.²⁸⁴ Gajdos et al.²⁸⁵ reported that in four of five patients given IGIV (1 to 2 gm/kg over 5 days), neurologic scores and vital capacity improved significantly. Fateh-Moghadam et al.²⁸⁶ noted improvement of the neurologic status of four patients with myasthenia gravis with a 7S IGIV preparation but not with a 5S IGIV preparation, implicating the Fc portion of the antibody molecule. Ippoliti et al.²⁸⁷ reported that six of seven patients given IGIV (400 mg/kg/day for 5 days) had significant neurologic improvement, which lasted at least 60 days in five of the six patients.

IGIV has been used in hemophilia with *auto-antibody to factor VIII*. In one patient in whom corticosteroids and vincristine were discontinued because of adverse effects, bimonthly IGIV (400 mg/kg/day for 5 days) resulted in significantly increased factor VIII levels that were maintained for 7 months after the infusions were stopped.²⁸⁸ In two other patients treated with IGIV, factor VIII levels increased and factor VIII inhibitor fell; repeated doses were necessary, however, before a stable increase in factor VIII levels was attained.²⁸⁹

IGIV was used in the treatment of 11 patients with biopsy-proven *bulbous pemphigoid*, a blistering skin disease associated with antiepidermal antibodies.²⁹⁰ Eight of nine patients given high-dose IGIV (400 mg/kg/day for 5 days) had a marked decrease in new blistering by the second day of therapy, but, except in one patient, effects did not last beyond 2 weeks. Two patients given lower doses showed no improvement.

The value of IGIV in *systemic lupus erythematosus* (SLE) is unproven. The platelet response to IGIV of patients with lupus-associated immune thrombocytopenia is similar to that of adults with thrombocytopenic purpura.²⁵⁷ Some but not all patients respond clinically to IGIV. In one patient, acute symptoms of SLE (rash, oral ulcer, fever, and pancytopenia) responded well, and remission was maintained with weekly IGIV infusions.²⁹¹ When the infusions were stopped 6 months later, the patient relapsed but again remitted on the reinstitution of IGIV therapy. In a series of 6 patients with SLE given IGIV, circulating immune complexes decreased, but C3 levels did not change.²⁹² Thus, in this limited experience, IGIV does not appear to be a promising therapeutic agent for SLE.

Combe and colleagues²⁹³ treated 31 patients with severe *rheumatoid arthritis* with human placenta eluted IGIV and measured a 50% improvement in 60% of patients. Four of six children with *newly diagnosed diabetes* treated with IGIV underwent prolonged partial remission.²⁹⁴ Because of evidence for autoimmune phenomena in *intractable childhood epilepsy*, Ariizumi and associates treated 16 patients with IGIV, resulting in complete clinical and EEG remission in four patients and marked clinical improvement in two.²⁹⁵ The validity of these isolated reports awaits larger controlled trials.

IGIV in Prematures and Newborns

The rationale for the use of IGIV in premature and newborn infants is based on their increased risk of infection, their incomplete acquisition of maternal antibodies, their sluggish antibody response to certain antigens, their physiologic hypogammaglobulinemia (particularly in the presence of profound prematurity), and their immature complement and phagocytic systems. Several recent studies indicate that IGIV may be of preventive or therapeutic value in certain high-risk, low-birth-weight infants. These are summarized in Table 15.

Sidiropoulos et al.²⁰⁷ treated successive infants suspected of neonatal

TABLE 15.
Possible Uses of Immune Globulin in Newborns and Infants*

Immune Globulin	Example
Intravenous	
Prevention of neonatal sepsis	Group B <i>Streptococcus</i>
Treatment of neonatal sepsis	With antibiotics, polymorphonuclear neutrophils
Prevention of neonatal viral disease	Adenovirus
Treatment of neonatal viral disease	Respiratory syncytial virus
Treatment of transient hypogammaglobulinemia of prematurity	
Oral	
Treatment of gastrointestinal viral disease	Rotavirus
Intramuscular	
Prevention of specific neonatal infections	Varicella, tetanus, hepatitis B†
Prevention of meningitis or sepsis	With bacterial polysaccharide immune globulin
Treatment of transient hypogammaglobulinemia of prematurity	

*From Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367. Used by permission.

†Proven efficacy.

sepsis with antibiotics plus IGIV (0.5 to 1.0 gm/day) for 6 days. Four of 15 (27%) of the antibiotic-treated group died compared to two of 20 (10%) of the antibiotic-IGIV group. A further breakdown indicated that four of nine (44%) infants less than 2,500 gm died compared to only one out of 11⁹ of infants given IGIV plus antibiotics; the latter is a statistically significant difference.

Haque et al.²⁹⁶ gave 50 normal Saudi Arabian newborns weighing less than 1,500 gm 125 mg/kg of IGIV on day 1 and another 50 newborns IGIV on days 1 and 8; 50 control infants got no treatment. Eight of 50 control infants got infected while four of 100 (4%) of the IGIV-treated children got infected. Since the rate of infection, even among the treated groups, was extremely high, this experience may not be relevant to a nursery population of a developed country.

The most extensive study conducted to date was carried out by Chirico et al.²⁹⁷ who gave alternate infants weighing less than 1,500 gm 0.5 gm/kg/week of IGIV for 4 weeks. They also gave IGIV weekly to alternate "high-risk" infants over 1,500 gm while they were on ventilators. Control infants got no IGIV. Thirty-one of 40 (77%) of the control infants less than 1,500 gm developed infections and 13 (32%) died. By contrast, 22 of 43 (51%) of IGIV-treated infants developed infection and 7 (16%) died. Among "high-risk" infants weighing less than 1,500 gm on ventilators, IGIV had no statistically significant benefit.

IGIV may also be effective in the treatment of certain neonatal viral infections. Prince and co-workers²³² showed that IGIV containing respiratory syncytial virus (RSV) antibody accelerated viral clearance in Cotton rats with established viral infections. A clinical trial in newborn RSV infection is underway. Treatment of established viral infection with IGIV has also been reported for echovirus 5, 7, 9, 11, adenovirus 7A, Argentine hemorrhagic fever, and CMV.²³¹ Newborns and immunodeficient and other immunocompromised patients are likely candidates for such experimental IGIV therapy.

IGIV could be used to prevent physiologic hypogammaglobulinemia of pregnancy. Morell and colleagues²⁹⁸ gave large (12 gm) of IGIV to mothers just before delivery and were able to significantly raise the neonatal IgG levels in infants over 3,200 gm. This approach was unsuccessful in infants less than 3,200 gm because of poor transplacental antibody passage. No one as yet has used repeated doses of IGIV during the first year of life such as was done with HISG by Amer and associates in 1963.⁹⁷ They gave ex-prematures monthly HISG injections for the first 8 months of life with minimal or no benefit; larger doses of IGIV might be more effective.

Future Directions

Several high titered human immune globulins are being tested for clinical efficacy indicating a polyvalent group B streptococcal immune globulin (for

newborns), a bacterial polysaccharide immune globulin (for high-risk infants), and a *Pseudomonas* immune globulin (for burn patients). High-dose antibody therapy is feasible by the use of the intravenous route.

Other potential therapeutic immune globulins include an *E. coli* endotoxin antibody (for shock or surgery), an anti-malarial antibody, and antiviral antibody such as echovirus or adenovirus.

Increased use of monoclonal antibodies to neutralize cells, bind to receptors, or detoxify certain drugs will be forthcoming. The use of monoclonal antibodies (even human monoclonal antibody) in specific therapy of certain infectious diseases may be forthcoming. Antiidiotypic antibodies may be of value in suppressing autoimmune disease or a specific harmful antibody.

The use of antibody directed to specific tumor cells or organ to deliver radioisotopes or chemotherapeutic agent is feasible. Such antibodies can also be used diagnostically for tumor cell localization.

References

1. Fibiger J: Om serum behandling af defteri. *Hospitalstidende* 1898; 6:337–339.
2. Babes V, Lepp M: Recherches sur la vaccination antirabique. *Ann Inst Pasteur* 1889; 3:385–390.
3. Cenci F: Alcune esperienze di sieroimmunizzazione e sieroterapia nel morbillo. *Riv Clin Pediatr* 1907; 5:1017–1025.
4. Debré R: Prevention de la coqueluche par l'injection de serum de coquelucheux preleve a la quatrieme semaine de la maladie. *Bull Acad Med Paris* 1923; 89:348–351.
5. Regan JC: Serum prophylaxis of epidemic parotitis. *JAMA* 1925; 84:279–280.
6. Stiehm ER: Passive immunization, in Feigen RC, Cherry JD: *Textbook of Pediatric Infectious Diseases*, ed 2. Philadelphia, WB Saunders Co, 1987, pp 2295–2319.
7. Bruton OC: Agammaglobulinemia. *Pediatrics* 1952; 9:722–728.
8. Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367.
9. Stiehm ER: The B lymphocyte system, in Stiehm ER, Fulginiti VA (eds): *Immunologic Disorders in Infants and Children*, ed 2. Philadelphia, WB Saunders Co, 1980, pp 52–81.
10. Medical Research Council Working Party: Hypogammaglobulinemia in the United Kingdom. *Lancet* 1969; 1:163–169.
11. Stiehm ER, Vaerman JP, Fudenberg HH: Plasma infusions in immunologic deficiency states: Metabolic and therapeutic studies. *Blood* 1966; 28:918–938.
12. Ellis EF, Henney CS: Adverse reactions following administration of human gammaglobulin. *J Allergy* 1969; 43:45–54.
13. Burks AW, Sampson HA, Buckley RH: Anaphylactic reactions after gamma globulin administration in patients with hypogammaglobulinemia. *N Engl J Med* 1986; 314:560–564.

14. Allen JC, Kunkel HG: Antibodies against gamma-globulin after repeated blood transfusions in man. *J Clin Invest* 1966; 45:29–39.
15. Stiehm ER, Fudenberg HH: Antibodies to gamma-globulin in infants and children exposed to isologous gamma-globulin. *Pediatrics* 1965; 35:229–235.
16. Fudenberg HH, Fudenberg BR: Antibody to hereditary human gamma-globulin (Gm) factor resulting from maternal-fetal incompatibility. *Science* 1964; 145:170.
17. Vyas GH, Perkins HA, Fudenberg HH: Anaphylactoid transfusion associated with anti-IgA. *Lancet* 1968; 2:312–315.
18. Björkander J, Hammarström L, Smith CIE, et al: Immunoglobulin prophylaxis in patients with antibody deficiency syndromes and anti-IgA antibodies. *J Clin Immunol* 1987; 7:8–15.
19. Stiehm ER, Fudenberg HH: Clinical and immunologic features of dysgammaglobulinemia type 1: Report of a case diagnosed in the first year of life. *Am J Med* 1966; 40:805–815.
20. Matheson DS, Clarkson TW, Gelfand EW: Mercury toxicity (acrodynia) induced by long-term injection of gamma globulin. *J Peds* 1980; 97:153–155.
21. Berger M, Cupps TR, Fauci A: Immunoglobulin replacement therapy by slow subcutaneous infusion. *Ann Intern Med* 1980; 93:55–56.
22. Welch MJ, Stiehm ER: Slow subcutaneous immunoglobulin therapy in a patient with reactions to intramuscular immunoglobulin. *J Clin Immunol* 1983; 3:285–286.
23. Park WH, Freeman RG Jr: The prophylactic use of measles convalescent serum. *JAMA* 1926; 87:556–558.
24. Zingher A: Convalescent whole blood, plasma and serum in prophylaxis of measles. *JAMA* 1924; 82:1180–1187.
25. Stillerman M, Marks HH, Thalhimer W: Prophylaxis of measles with convalescent serum. *Am J Dis Child* 1944; 67:1–14.
26. McKhann CF, Green AA, Coady H: Factors influencing the effectiveness of placental extract in the prevention and modification of measles. *J Pediatr* 1935; 6:603–614.
27. Stokes J Jr, Maris EP, Gellis SS: Chemical clinical and immunological studies on the products of human plasma fractionation: XI. The use of concentrated normal human serum in the prophylaxis and treatment of measles. *J Clin Invest* 1944; 23:531–540.
28. Ordman CW, Jennings CG Jr, Janeway CA: Chemical, clinical, and immunological studies on the products of human plasma fractionation: XII. The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles. *J Clin Invest* 1944; 23:541–549.
29. Greenberg M, Frant S, Rutstein DD: “Gamma globulin” and “placental globulin”: A comparison of their effectiveness in the prevention and modification of measles. *JAMA* 1944; 126:944–947.
30. Black FL, Yannet H: Inapparent measles after gamma globulin administration. *JAMA* 1960; 173:87–92.
31. Greenberg M, Pellitteri O, Eisenstein DT: Measles encephalitis: I. Prophylactic effect of gamma globulin. *J Pediatr* 1955; 46:642–647.
32. Krugman S, Giles JP, Jacobs AM, et al: Studies with live attenuated measles-virus vaccine. *Am J Dis Child* 1962; 103:353–363.

33. Stokes I Jr, Hilleman MR, Weibel RE, et al: Efficacy of live, attenuated measles virus vaccine given with human immune globulin. *N Engl J Med* 1961; 265:507-513.
34. Black FL, Berman LL, Libel M, et al: Inadequate immunity to measles in children vaccinated at an early age: Effect of revaccination. *Bull WHO* 1984; 62:315-319.
35. Stokes J Jr, Neefe JR: The prevention and attenuation of infectious hepatitis by gamma globulin. *JAMA* 1945; 127:144-145.
36. Havens WP Jr, Paul JR: Prevention of infectious hepatitis with gamma globulin. *JAMA* 1945; 129:270-271.
37. Gellis SS, Stokes J Jr, Brother GM, et al: The use of human immune serum globulin (gamma globulin) in infectious (epidemic) hepatitis in the Mediterranean theater of operations: I. Studies on prophylaxis in two epidemics of infectious hepatitis. *JAMA* 1945; 128:1062-1063.
38. Stokes J Jr, Farquhar JA, Drake ME, et al: Infectious hepatitis: Length of protection of immune serum globulin (gamma globulin) during epidemics. *JAMA* 1951; 147:714-719.
39. Ward R, Krugman S: Etiology, epidemiology, and prevention of viral hepatitis. *Prog Med Virol* 1962; 4:87-118.
40. Krugman S, Ward R, Giles JP, et al: Infectious hepatitis: Studies on the effectiveness of gamma globulin and on the incidence of inapparent infection. *JAMA* 1960; 174:823-830.
41. Woodson RD, Clinton JJ: Hepatitis prophylaxis abroad: Effectiveness of immune serum globulin in protecting Peace Corps volunteers. *JAMA* 1969; 209:1053-1058.
42. Hsia DY, Lonsway M Jr, Gellis SS: Gamma globulin in the prevention of infectious hepatitis: Studies on the use of small doses in family outbreaks. *N Engl J Med* 1954; 250:417-419.
43. Ward R, Krugman S, Giles JP, et al: Infectious hepatitis: Studies of its natural history and prevention. *N Engl J Med* 1958; 258:407-416.
44. Ward R, Krugman S: Etiology, epidemiology, and prevention of viral hepatitis. *Prog Med Virol* 1962; 4:87-118.
45. Krugman S: Effect of human immune serum globulin on infectivity of hepatitis. *J Infect Dis* 1976; 134:70-74.
46. Smallwood LA, Tabor E, Finlayson JS, et al: Antibodies to hepatitis A virus in immune serum globulin. *J Med Virol* 1981; 7:21-27.
47. Carl M, Francis DP, Maynard JE: Food-borne hepatitis A: Recommendations for control. *J Infect Dis* 1983; 148:1133-1135.
48. Grossman EB, Stewart SG, Stokes JP: Post-transfusion hepatitis in battle casualties. *JAMA* 1945; 129:991-994.
49. Duncan GG, Christian HA, Stokes J Jr: An evaluation of immune serum globulin as a prophylactic agent against homologous serum hepatitis. *Am J Med Sci* 1947; 213:53-57.
50. Drake ME, Barondess JA, Bashe WJ Jr, et al: Failure of convalescent gamma globulin to protect against homologous serum hepatitis. *JAMA* 1953; 152:690-693.
51. Holland PV, Rubenson RM, Morrow AG, et al: Gamma globulin in the prophylaxis of post-transfusion hepatitis. *JAMA* 1966; 196:471-474.
52. Grady GF: Prevention of post-transfusion hepatitis by gamma-globulin: Preliminary report. A cooperative study. *JAMA* 1970; 214:140-142.

53. Redeker AG, Mosley JW, Gocke DJ, et al: Hepatitis B immune globulin as a prophylactic measure for spouses exposed to acute type B hepatitis. *N Engl J Med* 1975; 293:1055–1059.
54. Kuhns WJ, Prince AM, Brotman B, et al: A clinical and laboratory evaluation of immune serum globulin from donors with a history of hepatitis: Attempted prevention of post-transfusion hepatitis. *Am J Med Sci* 1976; 272:255–261.
55. Torii A, Shoji N, Miyazoto Y: Presented at 10th meeting, International Society of Blood Transfusion, Stockholm, Sweden, 1964. Quoted in Mirick GS, et al: *N Engl J Med* 1965; 273:59–65.
56. Csapó J, Budai J, Bartos A, et al: Prevention of transfusion hepatitis. *Acta Paediatr Hung* 1963; 4:195–198.
57. Mirick GS, Ward R, McCollum RW: Modification of post-transfusion hepatitis by gamma globulin. *N Engl J Med* 1965; 273:59–65.
58. Katz R, Rodriguez J, Ward R: Post-transfusion hepatitis-effect of modified gamma globulin added to blood in vitro. *N Engl J Med* 1971; 285:925–932.
59. Gerety RJ, Smallwood LA, Tabor E: Hepatitis B immune globulin and immune serum globulin. *N Engl J Med* 1980; 303:329.
60. Perrillo RP, Campbell CR, Strang S, et al: Immune globulin and hepatitis B immune globulin: Prophylactic measures for intimate contacts exposed to acute type B hepatitis. *Arch Intern Med* 1984; 144:81–85.
61. Knodell RG, Ginsberg AL, Conrad AL, et al: Efficacy of prophylactic gamma globulin in preventing non-A, non-B post-transfusion hepatitis. *Lancet* 1:557.
62. Seeff LB, Zimmerman HJ, Wright EC, et al: A randomized, double-blind controlled trial of the efficacy of immune serum globulin for the prevention of post-transfusion hepatitis. *Gastroenterology* 1977; 72:111–121.
63. Simon N: Prevention of non-A, non-B hepatitis in haemodialysis patients by hepatitis B immunoglobulin. *Lancet* 1984; 2:1047.
64. Sugg U, Schneider W, Hoffmeister HE, et al: Hepatitis B immune globulin to prevent non-A, non-B post-transfusion hepatitis. *Lancet* 1985; 1:405–406.
65. Bodian D: Neutralization of three immunological types of poliomyelitis virus by human gamma globulin. *Proc Soc Exp Biol Med* 1949; 72:259–261.
66. Bodian D: Experimental studies on passive immunization against poliomyelitis: I. Protection with human gamma globulin against intramuscular inoculation and combined passive and active immunization. *Am J Hyg* 1951; 54:132–143.
67. Bloxsom A: Use of immune serum globulin (human) as prophylaxis against poliomyelitis. *Tex Med* 1949; 74:468–470.
68. Hammon W McD, Coriell LL, Stokes J Jr, et al: Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis (5 parts). *JAMA* 1954; 150:739–760, 1953; 151:1272–1285, 1954; 156:21–27.
69. Greenberg M: Gamma globulin in pediatrics. *Med Clin N Am* May 1947; 602–608.
70. Korns RF: Prophylaxis of German measles with immune serum globulin. *J Infect Dis* 1952; 90:183–192.
71. Grayston JT, Wattern RH: Epidemic rubella in Taiwan, 1957–1958: III. Gamma globulin in the prevention of rubella. *N Engl J Med* 1959; 261:1145–1150.
72. Brody JA, Sever JL, Schiff GM: Prevention of rubella by gamma globulin during an epidemic in Barrow, Alaska in 1964. *N Engl J Med* 1965; 272:127–129.

73. Houser HB, Schalet N: Prevention of rubella with gamma globulin. *Clin Res* 1958; 6:281–282.
74. Green RH, Balsamo MR, Giles JP, et al: Studies of the natural history and prevention of rubella. *Am J Dis Child* 1965; 110:348–365.
75. Krugman S, Ward R: Demonstration of neutralizing antibody in gamma globulin and re-evaluation of the rubella problem. *N Engl J Med* 1958; 259:16–19.
76. Lundstrom R, Thoren C, Blomquist G: Gamma globulin against rubella in pregnancy: I. Prevention of maternal rubella by gamma globulin and convalescent gamma globulin: A follow-up study. *Acta Paediatr* 1961; 50:444–452.
77. Lundstrom R, Thoren C, Blomquist B: Gamma globulin against rubella in pregnancy: II. Manifest maternal rubella in early pregnancy treated with convalescent gamma globulin: A follow-up study. *Acta Paediatr* 1961; 50:453–456.
78. Funkhouser WL: The use of gamma globulin antibodies to control chickenpox in a convalescent hospital for children. *J Pediatr* 1948; 32:257–259.
79. Schaeffer M, Toomey JA: Failure of gamma globulin to prevent varicella. *J Pediatr* 1948; 33:749–752.
80. Weintraub I: Treatment of herpes zoster with gamma globulin. *JAMA* 1955; 157:1611.
81. Lea WA Jr, Taylor WB: Gamma globulin in the treatment of herpes zoster. *Tex Med* 1958; 54:594–596.
82. Rodarte JG, Williams BH: Treatment of herpes zoster and chickenpox with immune globulin. *Arch Dermatol* 1956; 73:553–556.
83. Stevens DA, Merigan TC: Zoster immune globulin prophylaxis of disseminated zoster in compromised hosts. *Arch Intern Med* 1980; 140:52–54.
84. Ross AH: Modification of chickenpox in family contacts by administration of gamma globulin. *N Engl J Med* 1962; 267:369–376.
85. Iriarte PV, Tangco A, Jagasia KH, et al: Effect of gamma globulin on modification of chickenpox in children with malignant disease. *Cancer* 1965; 18:112–116.
86. Trimble GX: Attenuation of chickenpox with gamma globulin. *Can Med Assoc J* 1957; 77:697–699.
87. Brunell PA, Gershon AA: Passive immunization against varicella-zoster infections. *J Infect Dis* 1973; 127:415–423.
88. Brunell PA, Gershon AA, Hughes WT, et al: Prevention of varicella in high-risk children: A collaborative study. *Pediatrics* 1972; 50:718–722.
89. Thomas OC, McGovern JP: The gamma globulins with special reference to the controversy concerning their use for asthmatic children. *South Med J* 1964; 57:498–504.
90. Redner B, Markow H: Effects of minute doses of gamma globulin in children with active allergic manifestations. *JAMA* 1963; 185:692–695.
91. Abernathy RS, Strem EL, Good RA: Chronic asthma in childhood: Double-blind controlled study of treatment with gamma globulin. *Pediatrics* 1958; 21:980–993.
92. Hillman BC, Triplett F, Crawford LV, et al: Intracutaneous immune serum globulin therapy in allergic children. *JAMA* 1969; 207:902–906.
93. Baron S, Barnett EV, Goldsmith RS, et al: Prophylaxis of infections by gamma globulin. *Am J Hyg* 1964; 79:186–195.

94. Finkel KC, Haworth JC: Clinical trial to assess the effectiveness of gamma globulin in acute infections in young children. *Pediatrics* 1960; 25:798–806.
95. Mirick GS, Ward R, McCollum RW: Modification of post-transfusion hepatitis by gamma globulin. *N Engl J Med* 1965; 273:59–65.
96. Forman ML, Stiehm ER: Impaired opsonic activity but normal phagocytosis in low-birth-weight infants. *N Engl J Med* 1969; 281:926–931.
97. Amer J, Ott E, Ibbott FA, et al: The effect of monthly gamma globulin administration on morbidity and mortality from infection in premature infants during the first year of life. *Pediatrics* 1963; 32:4–9.
98. Diamond EF, Purugganan HB, Choi HJ: Effect of prophylactic administration on infection morbidity in premature infants. *IMJ* 1966; 130:668–670.
99. Steen JA: Gamma globulin in preventing infections in prematures. *Arch Pediatr* 1960; 77:291.
100. Kefalides NA, Arana JA, Bazan A, et al: Role of infection in mortality from severe burns: Evaluation of plasma, gamma globulin, albumin and saline solution therapy in a group of Peruvian children. *N Engl J Med* 1962; 267:317–323.
101. Stone HH, Graber CD, Martin JD Jr, et al: Evaluation of gamma globulin for prophylaxis against burn sepsis. *Surgery* 1965; 58:810–814.
102. Craig RDP: Immunotherapy for severe burns in children. *Plast Reconstr Surg* 1965; 35:263–270.
103. Nance FC, Hines JL, Fulton RE, et al: Treatment of experimental burn wound sepsis by post burn immunization with polyvalent *Pseudomonas* antigen. *Surgery* 1970; 68:248–253.
104. Cohen S, McGregor IA, Carrington S: Gamma globulin and acquired immunity to human malaria. *Nature* 1961; 192:733–737.
105. Kabat EA: Uses of hyperimmune human gamma globulin. *N Engl J Med* 1963; 269:247–254.
106. Craig RDP: Immunotherapy for severe burns in children. *Plast Reconstr Surg* 1965; 35:263–270.
107. Schless AP, Harell GS: Human gamma globulin in the treatment of bacterial infections. *Am J Med* 1968; 44:325–329.
108. Waisbren BA: The treatment of bacterial infections with the combination of antibiotics and gamma globulin. *Antibiot Chemother* 1957; 7:322–333.
109. Bodey GP, Nies BA, Mohberg NR, et al: Use of gamma globulin in infection in acute leukemia patients. *JAMA* 1964; 190:1099–1102.
110. Ziegler EJ, McCutchan JA, Fierer J, et al: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 1982; 307:122–123.
111. Centers for Disease Control: Postexposure prophylaxis of hepatitis B. *MMWR* 1984; 33:285–290.
112. Krugman S, Giles JP, Hammond J: Viral hepatitis type B (MS-2 strain): Prevention with specific hepatitis B immune serum globulin. *JAMA* 1971; 218:1665–1670.
113. Szmunes W, Prince AM, Goodman M, et al: Hepatitis B immune serum globulin in prevention of non-parenterally transmitted hepatitis B. *N Engl J Med* 1974; 290:701–706.
114. Seeff LB, Zimmerman HJ, Wright EC, et al: Efficacy of hepatitis B immune serum globulin following “needlestick” exposure: A preliminary report of the Veterans Administration Cooperative Study. *Lancet* 1975; 2:939–941.

115. Grady GF: Hepatitis B immune globulin prevention of hepatitis from accidental exposure among medical personnel. *N Engl J Med* 1973; 293:1067–1070.
116. Desmyter J, Bradburne AF, Vermynen C, et al: Hepatitis B immunoglobulin in prevention of HB, antigenemia in haemodialysis patients. *Lancet* 1975; 2:377–379.
117. Prince AM, Szmunes W, Mann MK, et al: Hepatitis B “immune” globulin: Effectiveness in prevention of dialysis-associated hepatitis. *N Engl J Med* 1975; 293:1063–1067.
118. Redeker AG, Mosely JW, Gocke DJ, et al: Hepatitis B immune globulin as a prophylactic measure for spouses exposed to acute type B hepatitis. *N Engl J Med* 1975; 293:1055–1059.
119. Beasley RP, Hwang LY, Stevens CE, et al: Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: Final report of a randomized double-blind, placebo-controlled trial. *Hepatology* 1983; 3:135–141.
120. Beasley RP, Lin CC, Wang KY, et al: Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. *Lancet* 1981; 2:388–392.
121. Jhaveri R, Rosenfeld W, Salazar D, et al: High titer multiple dose therapy with HBIG in newborn infants of HBsAg positive mothers. *J Pediatr* 1980; 97:305–308.
122. Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJT, et al: Prevention of chronic HBsAg carrier state in infants by HBsAg positive mothers by hepatitis B immunoglobulin. *Lancet* 1979; 2:436–438.
123. Wong VC, Ip HMH, Reesink HW, et al: Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis-B vaccine and hepatitis B immunoglobulin. *Lancet* 1984; 1:921–926.
124. Chung WK, Yoo JY, Sun HS, et al: Prevention of perinatal transmission of hepatitis B virus: A comparison between the efficacy of passive and passive-active immunization in Korea. *J Infect Dis* 1985; 151:280–286.
125. Kanai K, Takehiro A, Noto H, et al: Prevention of perinatal transmission of hepatitis B virus (HBV) to children of e antigen-positive HBV carrier mothers by hepatitis B immune globulin and HBV vaccine. *J Infect Dis* 1985; 151:287–290.
126. Tada H, Yanagida M, Mishina J, et al: Combined passive and active immunization for preventing perinatal transmission of hepatitis B virus carrier state. *Pediatrics* 1982; 70:613–619.
127. Szmunes W, Stevens CE, Oleszko WR, et al: Passive-active immunization against hepatitis B: Immunogenicity studies in adult Americans. *Lancet* 1981; 1:575–577.
128. Zchoval R, Jilg W, Lorbeer V, et al: Passive/active immunization against hepatitis B. *J Infect Dis* 1984; 150:112–117.
129. Acute Hepatic Failure Study Group: Failure of specific immunotherapy in fulminant type B hepatitis. *Ann Intern Med* 1977; 86:272–277.
130. Reed WD, Eddleston ALWF, Cullens H, et al: Infusion of hepatitis-B antibody in antigen-positive active chronic hepatitis. *Lancet* 1973; 2:1347–1351.
131. Bleyer AD: The use of immune blood in the treatment of whooping cough. *Am J Med Sci* 1917; 154:39–42.

132. Bradford WL: User of convalescent blood in whooping cough, with a review of the literature. *Am J Dis Child* 1935; 50:918-928.
133. Jundell I: Has specific serum of adults any value as a remedy against whooping cough? *Acta Paediatr* 1934; 16:1-11.
134. McGuiness AC, Gradford WL, Armstrong JG: The production and use of hyperimmune human whooping cough serum. *J Pediatr* 1940; 16:21-29.
135. Felton HM: The status of passive immunization and treatment in pertussis by the use of human hyperimmune serum. *JAMA* 1945; 128:26-28.
136. Kohn JL, Rudel F, Weichsel M, et al: Hyperimmune serum in treatment of whooping cough. *Am J Dis Child* 1947; 74:321-333.
137. Lucchesi PF, LaBocchetta AC: Whooping cough treated with pertussis immune serum (human). *Am J Dis Child* 1949; 77:15-24.
138. Ames RG, Cohen SM, Fischer AE, et al: Comparison of the therapeutic efficacy of four agents in pertussis. *Pediatrics* 1953; 11:323-337.
139. Morris D, McDonald JC: Failure of hyperimmune gamma globulin to prevent whooping cough. *Arch Dis Child* 1967; 32:236-239.
140. Bass JW, Klenk EL, Kotheimer JB, et al: Antimicrobial treatment of pertussis. *J Pediatr* 1969; 75:768-781.
141. Eichlseder W: Überprüfung der Wirksamkeit von Pertussis-Hyperimmun-Globulin and Chloramphenicol auf den Keuchhusten im Doppelten Blindversuch. *Arch Kinderheilk* 1963; 169:6-31.
142. Balagtas RC, Nelson KE, Levin S, et al: Treatment of pertussis with pertussis immune globulin. *J Pediatr* 1971; 79:203-208.
143. Habel K: Seroprophylaxis in experimental rabies. *Public Health Rep* 1945; 60:545-560.
144. Expert Committee on Rabies: Hyperimmune antirabies serum. *WHO Tech Rep Ser* 1950; 28:23-25.
145. Baltazard M, Bahmanyar M: Essai pratique du serum antirabique chez les mordus par loups enragés. *Bull WHO* 1955; 13:747-772.
146. Habel K, Koprowski H: Laboratory data supporting the clinical trial of anti-rabies serum in person bitten by a rabid wolf. *Bull WHO* 1955; 13:773-779.
147. Sikes RK: Human rabies immune globulin. *Public Health Rep* 1969; 84:797-801.
148. Lootbourow JC, Cabasso VJ, Roby RE, et al: Rabies immune globulin (human): Clinical trials and dose determination. *JAMA* 1971; 217:1825-1831.
149. Cabasso VJ, Loofbourow JC, Roby RE, et al: Rabies immune globulin of human origin: Preparation and dosage determination in non-exposed volunteer subjects. *Bull WHO* 1971; 45:303-315.
150. Wattanasri S, Boonthai P, Thongcharoen P: Human rabies after late administration of human diploid cell vaccine without hyperimmune serum. *Lancet* 1982; 2:870.
151. Shill M, Baynes RD, Miller SD: Fatal rabies encephalitis despite appropriate post-exposure prophylaxis. *N Engl J Med* 1987; 316:1257-1258.
152. Centers for Disease Control: Rabies prevention United States 1984: Recommendations of the Immunization Practices Advisory Committee. *MMWR* 1984; 33:393-402.
153. Patel JC, Mehta BC, Nanavati BH, et al: Role of serum therapy in tetanus. *Lancet* 1963; 1:740-743.
154. Brown A, Mohamed SD, Montgomery RD, et al: Value of a large dose of antitoxin in clinical tetanus. *Lancet* 1960; 2:227-230.

155. Lucas AO, Willis AJ, Mohamed SD, et al: A comparison of the value of 500,000 IU tetanus antitoxin with 200,000 IU in the treatment of tetanus. *Clin Pharmacol Ther* 1963; 6:592-597.
156. Vaishnava H, Goyal RK, Neogy CN, et al: A controlled trial of antiserum in the treatment of tetanus. *Lancet* 1966; 2:1371-1374.
157. Vakil BJ, Tulpule TH, Armitage P, et al: A comparison of the value of 200,000 IU tetanus antitoxin with 50,000 IU in the treatment of tetanus. *Clin Pharmacol Ther* 1963; 4:182-187.
158. Vakil BJ, Tulpule TH, Armitage P, et al: A comparison of the value of 200,000 IU tetanus antitoxin (horse) with 20,000 IU in the treatment of tetanus. *Clin Pharmacol Ther* 1964; 5:695-698.
159. Vakil BJ, Tulpule TH, Armitage P, et al: A comparison of the value of 200,000 IU of tetanus antitoxin (horse) with 10,000 IU in the treatment of tetanus. *Clin Pharmacol Ther* 1968; 9:465-471.
160. Athavale VB: Role of tetanus antitoxin in the treatment of tetanus in children. *J Pediatr* 1966; 68:289-293.
161. Rubinstein HM: Studies on human tetanus antitoxin. *Am J Hyg* 1962; 76:276-292.
162. Rubbo SD, Suri JC: Passive immunization against tetanus with human immune globulin. *Br Med J* 1962; 2:79-81.
163. McCracken GH Jr, Dowell DL, Marshall FN: Double-blind trial of equine antitoxin and human immune globulin in tetanus neonatorum. *Lancet* 1971; 1:1146-1149.
164. Blake PA, Feldman RA, Buchanan TM, et al: Serologic therapy of tetanus in the United States, 1965-1971. *JAMA* 1976; 235:42-44.
165. Gupta PS, Kapoor R, Goyal S, et al: Intrathecal human tetanus immunoglobulin in early tetanus. *Lancet* 1908; 2:439-440.
166. McComb JA, Dwyer RD: Passive-active immunization with tetanus immune globulin (human). *N Engl J Med* 1963; 268:857-862.
167. Hlava J, Honl I: Serum vaccinicum und seine Wirkungen. *Wien Klin Rundschau* 1895; 9:625-627.
168. Steinberg GM: Wissenschaftliche Untersuchungen uber das spezifische Infektionsagens der Blattern und die Erzeugung kunstlicher Immunitat gegen diese. *Krankheit Zentralbl Bakt I* 1896; 19:857-868.
169. Enders JF: Chemical, clinical and immunological studies on the products of human plasma fractionation: The concentrations of certain antibodies in globulin fractions derived from human blood plasma. *J Clin Invest* 1944; 23:510-530.
170. Verlinde JD, Spaander J: Neutralisatie van vaccine virus door gamma globuline. *Ned Tijdschr Geneesk* 1949; 93:2958-2962.
171. Gispén R, Lansberg HP, Nanning W: The effect of antivaccinia gamma globulin on smallpox vaccination in view of a proposed attempt to prevent post-vaccinal encephalitis. *Antonie van Leeuwenhoek* 1956; 22:89-102.
172. Barbero GJ, Gray A, Scott TFM, et al: Vaccinia gangrenosa treated with hyperimmune vaccinal gamma globulin. *Pediatrics* 1955; 16:609-618.
173. Kempe CH, Bowles C, Meiklejohn G, et al: The use of vaccinia hyperimmune gamma globulin in the prophylaxis of smallpox. *Bull WHO* 1961; 25:41-48.
174. Kempe CH, Berge TO, England B: Hyperimmune vaccinal gamma globulin: Source, evaluation, and use in prophylaxis and therapy. *Pediatrics* 1956; 18:177-188.

175. Kempe CH: Studies on smallpox and complications of smallpox vaccination. *Pediatrics* 1960; 26:176-189.
176. Lundstrom R: Complications of smallpox vaccination and their treatment with vaccinia immune globulin. *J Pediatr* 1956; 49:129-140.
177. Sharp JCM, Fletcher WB: Experience of antivaccinia immunoglobulin in the United Kingdom. *Lancet* 1973; 1:656-659.
178. Nanning W: Prophylactic effect of anti-vaccinia gamma globulin against post-vaccinial encephalitis. *Bull WHO* 1962; 27:317-324.
179. Brunell PA, Ross A, Miller LH, et al: Prevention of varicella by zoster immune globulin. *N Engl J Med* 1969; 280:1191.
180. Judelsohn RG, Meyers JD, Ellis RJ, et al: Efficacy of zoster immune globulin. *Pediatrics* 1974; 33:476-480.
181. Gershon AA, Steinberg S, Brunell PA: Zoster immune globulin: A further assessment. *N Engl J Med* 1974; 290:243-245.
182. Orenstein WA, Heymann DL, Ellis RJ, et al: Prophylaxis of varicella in high-risk children: Dose-response effect of zoster immune globulin. *J Pediatr* 1981; 98:368-373.
183. Zaia JA, Levin MJ, Preblud SR, et al: Evaluation of varicella-zoster immune globulin: Protection of immunosuppressed children after household exposure to varicella. *J Infect Dis* 1983; 147:737-743.
184. Paryani SG, Arvin AM, Koropchak CM, et al: Comparison of varicella-zoster antibody titers in patients given intravenous immune serum globulin or varicella-zoster immune globulin. *J Pediatr* 1984; 105:200-205.
185. Balfour HH Jr, Groth KE, McCullough J, et al: Prevention or modification of varicella using zoster immune plasma. *Am J Dis Child* 1977; 131:693-696.
186. Centers for Disease Control: Varicella-zoster immune globulin for the prevention of chickenpox: Recommendations of the Immunization Practices Advisory Committee. *Ann Intern Med* 1984; 100:859-865.
187. Apfelzweig R, Piskiewicz D, Hooper JA: Immunoglobulin A concentrations in commercial immunoglobulins. *J Clin Immunol* 1987; 7:46-50.
188. American Academy of Pediatrics: *Report of the Committee on Infectious Diseases*, ed 20. Evanston, Ill, 1986, pp 30-31.
189. Ammann AJ, Ashman RF, Buckley RH, et al: Use of intravenous gamma globulin in antibody immunodeficiency: Results of a multicenter controlled trial. *Clin Immunol Immunopathol* 1982; 22:60-67.
190. Ochs HD, Fischer SH, Virani FS, et al: Non-A, non-B hepatitis, and intravenous immune globulin. *Lancet* 1985; 1:404-405.
191. Lever AM, Webster ADB, Brown D, et al: Non-A, non-B hepatitis occurring in agammaglobulinaemic patients after intravenous immunoglobulin. *Lancet* 1984; 2:1062-1064.
192. Hornbrook MC, Dodd RY, Jacobs P, et al: Reducing the incidence of non-A, non-B post-transfusion hepatitis by testing donor blood for alanine aminotransferase. *N Engl J Med* 1982; 307:1315-1321.
193. Ochs HD, Fischer SH, Wedgwood RJ, et al: Comparison of high-dose and low-dose intravenous immunoglobulin therapy in patients with primary immunodeficiency diseases. *Am J Med* 1984; 76(3A):78-82.
194. Ochs HD, Buckley RH, Pirofsky B, et al: Safety and patient acceptability of intravenous immune globulin in 10% maltose. *Lancet* 1980; 2:1158-1159.
195. Cunningham-Rundles C, Siegal FP, Smithwick EM, et al: Efficacy of intra-

- venous immunoglobulin in primary humoral immunodeficiency disease. *Ann Intern Med* 1984; 101:435-439.
196. Pirofsky B: Intravenous immune globulin therapy in hypogammaglobulinemia: A review. *Am J Med* 1984; 76(3A):53-60.
 197. Schiff RI, Rudd C, Johnson R, et al: Use of a new chemically modified intravenous IgG preparation in severe primary humoral immunodeficiency: Clinical efficacy and attempts to individualize dosage. *Clin Immunol Immunopathol* 1984; 31:13-23.
 198. Pirofsky B, Campbell SM, Montanaro A: Individual patient variations in the kinetics of intravenous immune globulin administration. *J Clin Immunol* 1982; 2(suppl 2):7S-14S.
 199. Nolte MT, Pirofsky B, Gerritz GA, et al: Intravenous immunoglobulin therapy for antibody deficiency. *Clin Exp Immunol* 1979; 36:237-243.
 200. Cunningham-Rundles C, Smithwick EM, Siegal FP, et al: Use of intravenous pH 4.0 treated gamma globulin in humoral immunodeficiency disease, in Wedgwood RJ, Paul NW, Rosen FS (eds): *Primary Immunodeficiency Diseases*. New York, Alan R Liss, 1983, p 201.
 201. Montanaro A, Pirofsky B: Prolonged interval high-dose intravenous immunoglobulin in patients with primary immunodeficiency states. *Am J Med* 1984; 76(3A):67-72.
 202. Ashida ER, Saxon A: Home intravenous immunoglobulin by self-administration. *J Clin Immunol* 1986; 6:306-309.
 203. Kim KS: Efficacy of human immunoglobulin and penicillin G in treatment of experimental group B streptococcal infection. *Pediatr Res* 1987; 21:289-292.
 204. Kekomaki R, Eifenbein G, Gardner R, et al: Improved response of patients to random-donor platelet transfusions by intravenous gamma globulin. *Am J Med* 1984; 76(3A):199-203.
 205. Cross AS, Alving BM, Sadoff JC, et al: Intravenous immune globulin: A cautionary role. *Lancet* 1984; 1:912.
 206. Engelhard D, Warner JL, Kapoor N, et al: Fatal CMV infection associated with very high doses of intravenous immune globulin: A possible role of diminution of natural-killer cell activity, abstract 129. 4th International Symposium on Infections in the Immunocompromised Host, June 15-19, 1986, Ronneby Brunn, Sweden.
 207. Sidiropoulos D, Boehme U, Muralt GV, et al: Immunoglobulin supplementation in prevention or treatment of neonatal sepsis. *Pediatr Infect Dis* 1986; 5:S185-188.
 208. Austrian R, Gold J: Pneumococcal bacteremia with special reference to bacteremic pneumococcal pneumonia. *Ann Intern Med* 1964; 60:759-766.
 209. Farries JS, Dickson W, Greenwood E, et al: Meningococcal infection in Bolton, 1971-74. *Lancet* 1975; 2:118-120.
 210. Siber GR, Ambrosino DM, McIver J, et al: Preparation of human hyperimmune globulin to *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis*. *Infect Immunol* 1984; 45:248-254.
 211. Ambrosino D, Schreiber JR, Daum RS, et al: Efficacy of human hyperimmune globulin in prevention of *Haemophilus influenzae* type b disease in infant rats. *Infect Immunol* 1983; 39:709-714.
 212. Santosham M, Reid R, Ambrosino DM, et al: Prevention of *H. influenzae* type b infections in high-risk infants with bacterial polysaccharide immune globulin. *N Engl J Med* 1987; 923-927.

213. Winston DJ: Uses of intravenous immunoglobulin in viral infections, in Stiehm ER (moderator): Intravenous immunoglobulins as therapeutic agents. *Ann Intern Med* 1987; 107:367–382.
214. Winston DG, Ho WG, Lin CH, et al: Intravenous immunoglobulin for prevention of cytomegalovirus infection interstitial pneumonia after bone marrow transplantation. *Ann Intern Med* 1987; 106:12–18.
215. Snyderman DR, Werner BG, Heinze-Lacey B, CMVIG-IV Renal Transplant Study Group: Prevention of kidney transplant associated primary cytomegalovirus disease with an intravenous cytomegalovirus immune globulin: A preliminary analysis, abstract 843. *Program and Abstracts of the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC, American Society for Microbiology, 1985, p 248.
216. Condi RM, Hall BL, Howard RJ, et al: Treatment of life-threatening infections in renal transplant recipients with high-dose intravenous human IgG. *Transplant Proc* 1979; 11:66–68.
217. Nicholls AJ, Brown CB, Edward N, et al: Hyperimmune immunoglobulin for cytomegalovirus infections, letter. *Lancet* 1983; 1:532–533.
218. Blacklock HA, Griffiths P, Stork P, et al: Specific hyperimmune globulin for cytomegalovirus pneumonitis, letter. *Lancet* 1985; 2:152–153.
219. Reed EC, Bowden RA, Dandliker PS, et al: Efficacy of cytomegalovirus immune globulin in marrow transplant patients with cytomegalovirus pneumonia, abstract 731. *Program and Abstracts of the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington DC, American Society for Microbiology, 1986, p 231.
220. Lederman HM, Winkelstein JA: X-linked agammaglobulinemia: An analysis of 96 patients. *Medicine (Baltimore)* 1985; 64:145–156.
221. Crennan JM, Van Scoy RE, McKenna CH, et al: Echovirus polymyositis in patients with hypogammaglobulinemia: Failure of high-dose intravenous gammaglobulin therapy and review of the literature. *Am J Med* 1986; 81:35–42.
222. Prentice RL, Dagleish AG, Gatenby PA, et al: Central nervous system echovirus infection in Bruton's X-linked hypogammaglobulinemia. *Aust NZJ Med* 1985; 15:443–445.
223. Hadfield MG, Seidlin M, Houff SA, et al: Echovirus meningoencephalitis with administration of intrathecal immunoglobulin. *J Neuropathol Esp Neurol* 1985; 44:520–529.
224. Erlendsson K, Swartz T, Dwyer JM: Successful reversal of echovirus encephalitis in X-linked hypogammaglobulinemia by intraventricular administration of immunoglobulin. *N Engl J Med* 1985; 312:351–353.
225. Johnson PR Jr, Edward KM, Wright PF: Failure of intraventricular gamma globulin to eradicate echovirus encephalitis in a patient with X-linked agammaglobulinemia, letter. *N Engl J Med* 1985; 313:1546–1547.
226. Tobi M, Straus SE: Chronic Epstein-Barr virus disease: A workshop held by the National Institute of Allergy and Infectious Diseases. *Ann Intern Med* 1985; 103(6 pt 1):951–953.
227. Sullivan J: Personal communication, 1987.
228. Gammagard, immune globulin intravenous (human), product information monograph. Glendale, California, Travenol Laboratories, Inc, Therapeutic Division, 1985.
229. Gamimune N, immune globulin intravenous (human), 5% (in 10% maltose), pH 4.25, product information. Berkeley, California, Cutter Biological; 1984.

230. Sandoglobulin, immune globulin intravenous: Pharmacology and clinical studies, product information monograph. East Hanover, New Jersey, Sandoz, Inc, 1984.
231. Hemming VG, Prince GA: Intravenous immunoglobulin G in viral respiratory infections for newborns and infants. *Pediatr Infect Dis* 1986; 5(suppl 3):S204–206.
232. Prince GA, Hemming VG, Horswood RL, et al: Immunoprophylaxis and immunotherapy of respiratory syncytial virus infection in the cotton rat. *Virus Res* 1985; 3:193–206.
233. Hemming VG, Prince PG, Horswood RL, et al: Studies of passive immunotherapy for infections of respiratory syncytial virus in the respiratory tract of a primate model. *J Infect Dis* 1985; 152:1083–1087.
234. Prince GA, Hemming VG, Chanock RM: The use of purified immunoglobulin in the therapy of respiratory syncytial virus infection. *Pediatr Infect Dis* 1986; 5(suppl 3):S201–203.
235. Dagan R, Schwartz RH, Insel RA, et al: Severe diffuse adenovirus 7a pneumonia in a child with combined immunodeficiency: Possible therapeutic effect of human serum globulin containing specific neutralizing antibody. *Pediatr Infect Dis* 1984; 3:246–251.
236. Stiehm ER, Chin TW, Haas A, et al: Infectious complications of the primary immunodeficiencies. *Clin Immunol Immunopathol* 1986; 40:69–86.
237. Losonsky G, Johnson JP, Winkelstein JA, et al: Oral administration of human serum immunoglobulin in immunodeficient patients with viral gastroenteritis: A pharmacokinetic and functional analysis. *J Clin Invest* 1985; 76:2362–2367.
238. Barnes GL, Doyle LW, Hewson PH, et al: A randomised trial of oral gammaglobulin in low-birth-weight infants infected with rotavirus. *Lancet* 1982; 1:1361–1363.
239. Calvelli TA, Rubinstein AR: Intravenous gamma-globulin in infant acquired immunodeficiency syndrome. *Pediatr Infect Dis* 1986; 5(suppl 3):S207–210.
240. Centers for Disease Control: Safety of therapeutic immune globulin preparations with respect to transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus infection. *MMWR* 1986; 35:231–233.
241. Wood CC, Williams AE, McNamara JC, et al: Antibody against the human immunodeficiency virus in commercial intravenous gammaglobulin preparations. *Ann Intern Med* 1986; 105:536–538.
242. Wells MA, Wittek AE, Epstein JS, et al: Inactivation and partition of human T-cell lymphotropic virus, type III, during ethanol fractionations of plasma. *Transfusion* 1986; 26:210–213.
243. Imbach P, d'Apuzzo V, Hirt A: High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet* 1981; 1:1228–1230.
244. Imbach P, Barundun S, Baumgartner C, et al: High-dose intravenous gammaglobulin therapy of refractory purpura in particular idiopathic thrombocytopenia in childhood. *Helv Paediatr Acta* 1981; 36:81–86.
245. Bussel JB, Kimberly RP, Inman RD, et al: Intravenous gammaglobulin treatment of chronic idiopathic thrombocytopenic purpura. *Blood* 1983; 62:480–486.
246. Uchino H, Yasunaga K, Akatsuka JI: A cooperative clinical trial of high-dose immunoglobulin therapy in 177 cases of idiopathic thrombocytopenic purpura. *Thromb Haemost* 1984; 51:182–185.

247. Bussel JB, Goldman A, Imbach P, et al: Treatment of acute idiopathic thrombocytopenia of childhood with intravenous infusions of gammaglobulin. *J Pediatr* 1985; 106:886–890.
248. Imbach P, Berchtold W, Hirt A, et al: Intravenous immunoglobulin versus oral corticosteroids in acute immune thrombocytopenic purpura in childhood. *Lancet* 1985; 2:464–468.
249. Bussel JB: Treatment of acute idiopathic thrombocytopenic purpura. *J Pediatr* 1986; 108:326–327.
250. Bussel JB, Schulman I, Hilgartner MW, et al: Intravenous use of gammaglobulin in the treatment of chronic immune thrombocytopenic purpura as a means to defer splenectomy. *J Pediatr* 1983b; 103:651–653.
251. Mori PG, Mancuso G, Del Principe D, et al: Chronic idiopathic thrombocytopenia treated with immunoglobulin. *Arch Dis Child* 1983; 58:851–855.
252. Nydegger UE, Spycher MO: Polyclonal polyspecific antibody mixtures from random donors as therapeutic tools in autoimmune disease, in Morey A, Nydegger UE (eds): *Clinical Use of Intravenous Immunoglobulins*. London, Academic Press, 1986, pp 431–441.
253. Bussel J, Pahwa S, Porges A, et al: Correlation of in vitro antibody synthesis with the outcome of intravenous γ -globulin treatment of chronic idiopathic thrombocytopenic purpura. *J Clin Immunol* 1986; 6:50–56.
254. Ball S, Zuible A, Roster BLT, et al: Changes in platelet immunoprotein levels during therapy in adult immune thrombocytopenia. *Br J Haematol* 1985; 60:631–633.
255. Stohl W: Cellular mechanisms in the in vitro inhibition of pokeweed mitogen-induced B cell differentiation by immunoglobulin for intravenous use. *J Immunol* 1986; 136:4407–4413.
256. Gross B, Haessig A, Luescher EF, et al: Monomeric IgG preparations for intravenous use inhibit platelet stimulation by polymeric IgG. *Br J Haematol* 1983; 53:289–299.
257. Bussel JB, Hilgartner MW: The use and mechanism of action of intravenous immunoglobulin in the treatment of immune haematologic disease. *Br J Haematol* 1984; 56:1–7.
258. Becton DL, Kinney TR, Chaffee S, et al: High-dose intravenous immunoglobulin for severe platelet alloimmunization. *Pediatrics* 1984; 73:1120–1123.
259. Junghans RP, Ahn YS: High-dose intravenous gamma globulin to suppress alloimmune destruction of donor platelets. *Am J Med* 1984; 76(3A):204–208.
260. Berney SI, Metcalfe P, Wathen NC, et al: Post-transfusion purpura responding to high dose intravenous IgG: Further observations on pathogenesis. *Br J Haematol* 1985; 61:627–632.
261. Viero P, Cortelazzo S, Buelli M, et al: Thrombotic thrombocytopenic purpura and high-dose immunoglobulin treatment, letter. *Ann Intern Med* 1986; 104:282.
262. Chirico F, Duse M, Ugazio AG: High-dose intravenous gammaglobulin therapy for passive immune thrombocytopenia in the neonate. *J Pediatr* 1983; 103:654–655.
263. Tchernia G, Dreyfus N, Laurian Y, et al: Management of immune thrombocytopenia in pregnancy: Response to infusions of immunoglobulins. *Am J Obstet Gynecol* 1984; 148:225–226.

264. Wenske C, Gaedicke G, Kuenzlen E, et al: Treatment of idiopathic thrombocytic purpura in pregnancy by high-dose intravenous immunoglobulin. *Blut* 1983; 46:347–353.
265. Davies SV, Murray JA, Gee H, et al: Transplacental effect of high-dose immunoglobulin in idiopathic thrombocytopenia (ITP), letter. *Lancet* 1986; 1:1098–1099.
266. Massey GV, McWilliams NB, Mueller DG, et al: Intravenous immunoglobulin in neonatal isoimmune thrombocytopenia. *J Pediatr* 1987; 111:133–135.
267. Mueller-Eckhardt C, Kuenzlen E, Thilo-Korner D, et al: High-dose intravenous immunoglobulin for post-transfusion purpura, letter. *N Engl J Med* 1983; 308:278.
268. Goldstein R, Blanchette VS, Huebsch LB, et al: Treatment of gold-induced thrombocytopenia by high-dose intravenous gamma globulin. *Arthritis Rheum* 1986; 29:426–430.
269. DiTuro WJ, Goldsmith PM, Frenkel ID, et al: Intravenous gammaglobulin treatment of profound herpes varicella zoster associated thrombocytopenia. *Ann Allergy* 1986; 56:206, 241–243.
270. Ordi J, Vilardell M, Alijotas J, et al: Serum thrombocytopenia and high-dose immunoglobulin treatment, letter. *Ann Intern Med* 1986; 104:282–283.
271. Pollack S, Cunningham-Rundles C, Smithwick EM, et al: High-dose intravenous gamma globulin for autoimmune neutropenia, letter. *N Engl J Med* 1982; 307:253.
272. Bussel JB, LaLezari P, Hilgartner M, et al: Reversal of neutropenia with intravenous gammaglobulin in autoimmune neutropenia of infancy. *Blood* 1983; 62:398–400.
273. Engelhard D, Waner JL, Kapoor N, et al: Effect of intravenous immune globulin on natural killer cell activity: Possible association with autoimmune neutropenia and idiopathic thrombocytopenia. *J Pediatr* 1986; 108:77–81.
274. MacIntyre EA, Linch DC, Macey MG, et al: Successful response to intravenous immunoglobulin in autoimmune haemolytic anaemia. *Br J Haematol* 1985; 60:387–388.
275. Oda H, Honda A, Sugita K, et al: High-dose intravenous intact IgG infusion in refractory autoimmune hemolytic anemia (Evans syndrome). *J Pediatr* 1985; 107:744–746.
276. Koren G, Lavi S, Rose V, et al: Kawasaki disease: Review of risk factors for coronary aneurysms. *J Pediatr* 1986; 108:388–392.
277. Ammann AJ, Wara DW: Collagen vascular diseases, in Rudolph A, Hoffman J (eds): *Pediatrics*. Norwalk, Connecticut, Appleton, Century Crofts, 1982, pp 443–444.
278. Neches WH: Kawasaki disease: A continuing puzzle. *Int J Cardiol* 1986; 11:1–7.
279. Melish ME: Intravenous immunoglobulin in Kawasaki syndrome: A progress report. *Pediatr Infect Dis* 1986; 5:S211–215.
280. Furusho K, Kamiya T, Nakano H, et al: High-dose intravenous gammaglobulin for Kawasaki disease. *Lancet* 1984; 2:1055–1058.
281. Newburger JW, Takahashi M, Burns JC, et al: The treatment of Kawasaki syndrome with intravenous gamma globulin. *N Engl J Med* 1986; 315:341–347.
282. Nagashima M, Matsushima M, Matsuoka H, et al: High dose gamma globulin therapy for Kawasaki disease. *J Pediatr* 1987; 110:710–712.

283. Lueng DYM, Burns JC, Newburger JW, et al: Reversal of lymphocyte activation in vivo in the Kawasaki syndrome by intravenous gamma globulin. *J Clin Invest* 1987; 79:468-472.
284. Hermann C Jr, Lindstrum JM, Kessey JC, et al: Myasthenia gravis-current concepts (clinical conference). *West J Med* 1985; 142:797-809.
285. Gajdos P, Outin H, Elkharrat D, et al: High-dose intravenous gammaglobulin for myasthenia gravis, letter. *Lancet* 1984; 2:406-407.
286. Fateh-Moghadam A, Wick M, Besinger U, et al: High-dose intravenous gammaglobulin for myasthenia gravis. *Lancet* 1984; 1:848-849.
287. Ippoliti G, Cost V, Piccolo G, et al: High-dose intravenous gammaglobulin for myasthenia gravis, letter. *Lancet* 1984; 2:809-810.
288. Gianella-Borradori A, Hirt A, Luthy A, et al: Haemophilia due to factor VIII inhibitors in a patient suffering from an autoimmune disease: Treatment with intravenous immunoglobulin. *Blut* 1984; 48:403-407.
289. Rossi F, Sultan Y, Kazatchkine MD: Spontaneous and therapeutic suppression of autoimmune response to factor VIII by anti-idiotypic antibodies, in Morrel A, Nydegger UE (eds): *Clinical Use of Intravenous Immunoglobulins*. London, Academic Press, 1986, pp 421-430.
290. Godard W, Roujeau JC, Guillot B, et al: Bullous pemphigoid and intravenous gammaglobulin, letter. *Ann Intern Med* 1986; 103:(6 pt 1):964-965.
291. Gaedicke G, Teller WM, Kohne E, et al: IgG therapy in systemic lupus erythematosus: Two case reports. *Blut* 1984; 48:387-390.
292. Lin RY, Racis SP: In vivo reduction of circulating C1q binding immune complexes by intravenous gammaglobulin administration. *Int Arch Allergy Appl Immunol* 1986; 79:286-290.
293. Combe B, Cosso B, Clot J, et al: Human placenta-eluted gammaglobulins in immunomodulating treatment of rheumatoid arthritis. *Am J Med* 1985; 78:920-928.
294. Heinze E, Thon A, Vetter U, et al: Gamma-globulin therapy in six newly diagnosed diabetic children. *Acta Paediatr Scand* 1985; 74:605-606.
295. Ariizumi M, Baba K, Shiihara H, et al: High dose gammaglobulin for intractable childhood epilepsy, letter. *Lancet* 1983; 2:162-163.
296. Haque KN, Zaidi MH, Haque SK, et al: Intravenous immunoglobulin for prevention of sepsis in preterm and low birthweight infants. *Pediatr Infect Dis* 1986; 5:622-625.
297. Chirico G, Rondini G, Plebani A, et al: Intravenous gammaglobulin therapy for prophylaxis of infection in high-risk neonates. *J Pediatr* 1987; 110:437-424.
298. Morell A, Sidiropoulos D, Herrmann U, et al: IgG subclasses and antibodies to group B streptococci in preterm neonates after intravenous infusion of immunoglobulin to the mothers. *Pediatr Infect Dis* 1986; 5:S195-197.

Pathophysiology of Childhood Obesity*

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Obesity is one of the most prevalent nutritional problems in modern western societies. Depending upon the diagnostic criteria employed, as many as 25% of the children and 30% of the adult population in the United States are obese.¹⁻⁵ The persistence of juvenile-onset obesity into adulthood may create the metabolic groundwork for various degenerative cardiovascular disturbances of later life. Partly because adiposity-associated morbidities (hyperinsulinism, hypertension, hyperlipidemia, etc.) are often not evident until adulthood, the medical and allied professions have not given pediatric obesity the attention it deserves.

Citizens of the United States spend over 10 billion dollars annually on books, drugs, special nutrient preparations, and other regimens designed to produce weight loss.⁶ Approximately 30 million dollars per annum are spent on obesity-related research by the National Institutes of Health.⁷ Clinicians need little reminding of the intractable nature of this disorder and of the need for both effective prevention and more long-lasting modes of intervention. While obesity in children must be recognized as a serious disease entity, with significant implications for adult morbidity and mortality, the imposition of ineffective treatments and societal stereotypes on the obese child may be equally damaging. This chapter will familiarize the reader with current knowledge regarding the etiology, epidemiology, morbidity, therapy, and prevention of obesity.

Diagnostic Criteria for Obesity

Definition

Obesity may be defined as a maladaptive increase in the size of the adipose organ. An individual's optimal amount of adipose tissue relative to

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lean body mass (muscle, liver, brain, etc.) is dependent on factors such as age, intercurrent health, genotype, and environment. It was probably not maladaptive, for example, for our distant progenitors to be generously endowed with adipose tissue since they were often required to survive prolonged periods of starvation.⁸ Similarly, there are physiologic states, such as early pregnancy, when it may be advantageous to have more energy stored as fat.⁹

All current anthropometric techniques for defining "obesity" in children employ actuarial approaches to its diagnosis. A specific weight-for-height or skinfold thickness, corrected for age and sex, is defined as "abnormal" on a statistical (versus population norms) rather than a functional (versus risk of associated illness) basis. The former approach is reasonable at present because we do not yet possess the means to predict either the likelihood of temporal persistence of a given body composition or its probability of causing illness. In adults, where the association between body composition and medical morbidity are clearer (see section on adipose tissue distribution), risk assessment can be made with greater precision. Even in the case of adults, however, predictions regarding future morbidity related to body composition (or fat distribution) are made on an actuarial and not an individual basis, that is, we are not yet able to make prognostications that take account of specific genetic or environmental variables. These points should be recalled when considering the diagnosis of "obesity" in an individual child or adult.

Any objective means of quantifying adiposity should ideally yield information regarding current and/or subsequent risk of medical morbidity. However, the degree of adiposity likely to provoke such manifestations varies from individual to individual.

Adiposity cannot be defined by weight alone, since weight measures are confounded by height and do not distinguish lean body mass (all tissue that is not fat) from adipose tissue mass. Ideally, any clinical means of quantifying adiposity should possess the following attributes:

1. Easily performed in an outpatient setting.
2. Not be confounded by other body mass covariates such as height.
3. Yield reproducible results that can be matched against available reference standards.
4. Correlate with present and/or future adiposity-related morbidity.
5. Provide information regarding anatomic distribution of adipose tissue (see section on adipose tissue distribution).

This section reviews some of the more commonly used methods of assessing fatness. For reasons discussed earlier, none of these techniques satisfies all of the aforementioned criteria.

Assessment of Body Composition

Densitometry.—Densitometry has long been regarded as the "gold standard" against which other methods of estimating body composition

should be rated.^{10, 11} Briefly, this technique treats the body as if it were composed of two compartments: adipose tissue (AT) and lean body mass (LBM). It is assumed that body density is a proportional function of the density of LBM (1.1 gm/ml) and the density of AT (0.9007 gm/ml), and that the densities of these two compartments remain constant throughout life and do not vary between individuals. Differences in body density determined by hydrostatic (underwater) weighing as compared with weight in air can then be used to estimate the relative proportions of these two compartments.^{10, 12, 13} While the assumption that the mean densities of these two compartments remain relatively constant is apparently valid in adults, it may not be so for children. Total body water in children, expressed as a percentage of total body mass, declines by as much as 5% to 10% from infancy to adolescence, while the concentration of minerals in bone increases more than twofold from infancy to adulthood. Similarly, the potassium and protein concentrations in muscle are not constant during growth. These factors combine to produce a lower density of LBM in children than adults, thus mooted to some extent the applicability of this difficult procedure to the pediatric population until more data are available on body composition during growth.^{12, 14-21}

Total Body Electrical Conductivity.—Total body electrical conductivity (TOBEC) has recently been described as a means of assessing lean body mass that is independent of the assumptions regarding body density that currently limit the use of densitometry in children.^{22, 23} TOBEC is based on the principle that body water content can be assayed indirectly by measuring the conductance of the human body when placed in an electromagnetic field. Subjects are introduced into a solenoidal coil that is driven by a 5-MHz oscillating frequency current. Changes in coil impedance are proportional to the electrical conductivity of the body. Electrical conductivity is proportional to ion-containing body water (i.e., LBM). TOBEC is noninvasive and correlations of lean body mass measured by TOBEC and by hydrodensitometry in adults and children are good.²⁴ LBM as determined by TOBEC does not appear to be affected by variation in the water and potassium content of LBM in adults and therefore is probably not affected by changes in these parameters that occur during growth.¹¹ Once LBM is assessed, AT mass can be determined by subtracting LBM from total body weight. However, despite its excellent correlation with densitometric measures, TOBEC requires expensive equipment that is not readily available to the pediatrician. Population profiles of normative data for LBM based on TOBEC are not yet available.

Weight/Height Ratios.—The most commonly used clinical indices of adiposity are various mathematical permutations of the ratio of weight to height. These manipulations are designed to generate a relationship in which height is eliminated as a significant covariate of weight. The body mass index (weight)/(height in meters)² = BMI is highly correlated with densitometric assessment of body fat and poorly correlated with height.²⁵⁻²⁷ Normative data for BMI percentiles in the USA based on age, sex, and

ethnicity are available.²⁸ While BMI represents a useful parameter, it may not detect children at risk for adiposity-related morbidities due to fat distribution (see section on adipose tissue distribution) and will "mislabel" muscular children as obese.

Skinfold Thickness.—The most accurate of the readily available and standardized methods of assessing adiposity is the direct measure of subcutaneous fat at various anatomic sites using skinfold calipers. Variability can be reduced to less than 3% if measurements are taken repeatedly (three to five times) by an experienced operator.^{13, 29–32} Normative values for skinfold thickness percentiles at various sites (triceps, subscapular, etc.) reflecting age, sex, and ethnicity are available.^{28, 33, 33–39} Correlation with densitometric assessment of body fat is good.⁴⁰ While some training is required to become proficient in this method, its benefits probably outweigh the inconvenience, and skinfold thickness measurement should be included in the routine evaluation of the growing child.

In summary, densitometry is regarded as the most accurate means of assessing total body fat, but relies on assumptions regarding the composition of LBM that may not be valid in children. Both BMI and skinfold thickness have been shown to correlate highly with other measures of total body fat, including densitometry and computed tomography.^{19, 41, 42} While BMI is more easily determined and is more reproducible than skinfold thickness, the latter yields more information and is better correlated with adiposity-related morbidities (see section on adipose tissue distribution). Other methods may prove superior once more reference data and experience have been reported, but at present skinfold thicknesses appears to be the best readily available means of assessing body composition and AT distribution in children.

Basic Physiology of Body Weight Regulation

The quantity of AT in the body reflects the cumulative balance between energy intake and output. Obesity is ultimately the result of an excess of energy intake relative to energy expenditure. In this sense, excessive adiposity is a reflection of the activities of systems controlling energy homeostasis rather than a primary abnormality of fat. Regarding a specific obese individual, however, no a priori judgement can be made as to whether there is a disorder of energy intake, output, or both.

If energy intake and utilization were independent processes, then the prediction of change in body fat in response to dietary manipulation or change in energy expenditure would be comparatively simple. Any change in either of these parameters would result in a corresponding weight change of approximately 1 gm of fat per 7 kcal alteration in intake relative to expenditure. By taking in 150 calories (an 8-oz glass of milk) more or less per day than energy output, an individual's body weight would then change by 21.4 gm per day or 7.8 kg per year. However, energy intake,

metabolic efficiency (calories stored per calories consumed), and body mass are interrelated variables whose interlocking control mechanisms are poorly understood. The relative long-term constancy of body composition in adults, and the metabolic changes that accompany the weight-altered state, strongly suggest that body mass is somehow regulated by complex homeostatic mechanisms.⁴³⁻⁴⁷ This section reviews the evidence that body weight is regulated as well as some of the possible cybernetics of feedback loops that may influence energy balance.

Energy Intake

Feeding behavior in animals is regulated by a complex set of internal and external cues that "inform" the organism when to initiate feeding, what volume and/or calories to consume, and when to stop feeding. Some instances of human obesity may be due to primary abnormalities in this regulatory system.

Classically, investigators of central nervous mechanisms regulating feeding behavior have viewed the brain as having discrete hunger and satiety "centers" located respectively in the lateral hypothalamus (LH) and ventromedial hypothalamus (VMH).⁴⁸⁻⁵⁰ Signal output from these regions is influenced by various neurotransmitters, circulating metabolites, and hormones.⁵¹⁻⁵⁶ Since other brain regions (e.g., the paraventricular nucleus and hindbrain), as well as widely distributed fiber tracts, also influence feeding behavior,⁵⁷⁻⁷⁵ these hypothalamic areas are more accurately viewed as components of central hunger and satiety "systems" that regulate feeding behavior via an interplay of central and peripheral pathways (Table 1).

Numerous other modulators of feeding behavior have been identified, including thalamic opiate and nonopiate reward systems,⁵⁶ central and hepatic glucoreceptors,^{76, 77} and various gut peptides (Table 2).⁷⁸ Afferents to central feeding control regions consist of active or permissive neurosensory (smell), metabolic (cholecystokinin), and hormonal (insulin, glucose, etc.) cues.^{56, 79-106} The efferent pathways from these areas include vagal and sympathetic nerve tracts as well as various neuroendocrine axes, such as those controlling the thyroid and adrenal glands.⁴⁷ These systems, in turn, influence glucose utilization, insulin release, thermogenesis, and other energy homeostatic mechanisms including food intake.^{49, 107-114}

Pharmacologic, surgical, and electrophysiologic studies of these systems have raised important issues regarding feeding behavior and body weight regulation. Some investigators have suggested that lesions of (or in the regions of) the VMH or LH alter a "set point" for body weight. The set point for body weight is a theoretical construct that may be operationally defined as that body weight or composition that the organism "defends" by varying ingestive behavior or systemic metabolic efficiency (or both) over long periods of time.¹¹⁵⁻¹¹⁷ Alternatively, others have proposed that

TABLE 1.
Regulation of Feeding Behavior via the Hypothalamus

	Lateral Hypothalamus	Ventromedial Hypothalamus
Effects of hypothalamic stimulation and destruction on feeding and metabolism in animals		
Stimulation	Hunger, glycogen synthesis	Satiety, glycogenolysis
Destruction	Hypophagia, hypometabolic state	Hyperphagia, hypersensitivity to pleasant and unpleasant food cues, hyperinsulinism
Effects of different inputs on hypothalamic electrical activity in animals	↑ or ↓	
Food cues	None	None
Cholecystokinin	None	↑
Neurotensin	None	↑
Galanin	None	↓
Neuropeptide Y	None	↓
α-Adrenergic	None	↓
β-Adrenergic	↓	None
Serotonin	None	↓
Dopamine	↓	None
Opioid	None	↓

TABLE 2.
The Effect of Different Agents on Food Intake*

Agents Increasing Food Intake	Agents Decreasing Food Intake
Urine from hyperphagic animals ⁸⁶	Urine from anorexic animals ⁸⁶
Nonmetabolisable glucose analogs ^{91, 92}	Blood from satiated animals ⁸⁸
Insulin ^{66, 94}	Glucose and glycerol ^{76, 83, 93, 100}
Androgens ⁶⁹	Insulin ⁶⁴
Glucocorticoids ^{105, 106}	Estrogens ⁷³
Caffeine ⁷¹	Prostaglandins ^{101, 102}
Barbiturates ⁶⁰	Cholecystokinin ^{74, 78, 81}
5-Hydroxytryptophan antagonists ^{65, 66}	Amphetamines ⁷¹
Norepinephrine and epinephrine (α -effects) ^{52, 53}	5-Hydroxytryptophan ⁷²
Opiate agonists ^{56, 67, 84}	Norepinephrine and epinephrine (β -effects) ^{52, 56}
Neuroleptics ^{61, 63, 64}	Naloxone ^{75, 83}
Neuropeptide Y ^{97, 98}	Serotonin ^{66, 72, 90}
Clonidine ¹⁰³	Atropine ⁷⁰
	Somatostatin ¹⁰⁴
	Glucagon ^{95, 96}

*Note that insulin, norepinephrine, and epinephrine can stimulate or inhibit feeding behavior. Insulin may be secreted in response to various food cues and may then directly or indirectly facilitate the initiation of feeding. The rise in circulating insulin levels that occurs following nutrient ingestion may promote the termination of feeding. The mixed α - and β -adrenergic activity of norepinephrine and epinephrine influences signals in tracts to the VMH as well as the LH and can also be involved in both meal initiation and termination. It is clear that there is no single pathway or neurotransmitter that regulates feeding behavior. It is, instead, a complex interplay of many systems.

the effects of such brain lesions on body mass are due to changes in the activity of neural outflow tracts, such as vagally mediated insulin release^{57-59, 118, 119} or sensitivity to aversive as well as pleasant external and internal stimuli.¹²⁰⁻¹²³ The latter hypotheses do not require that the organism receive central inputs regarding body mass or possess any type of internal set point (see section on energy expenditure for further discussion of set-point theories).

Bray¹²⁴ has hypothesized that the intake and metabolism of carbohydrates, proteins, and fats may each be regulated by nutrient-specific feedback mechanisms. Drewnowski et al.¹²⁵ measured taste sensitivity and preference for sweetness, lipid content, and creaminess in food in obese, reduced-obese, and never-obese subjects. They found that ability to detect

the relative composition of different formulas was equal in all subject groups. However, the obese individuals exhibited a greater taste preference for foods with higher fat content (34% lipid, 5% sucrose) than lean controls (20% lipid, 10% sucrose). Reduced obese subjects exhibited a greater taste preference for fattier foods (35% lipid) than lean controls and for sweeter foods (10.1% sucrose) than obese subjects. The authors hypothesize that sensory preference for dietary fats and sugars is influenced by body weight status. These data also suggest that the formerly obese individual retains a preference for calorically dense fatty foods, even when obesity is no longer present.

Obese infants have been reported to consume significantly more sweetened formula than lean infants, which is in agreement with the hypothesis that obese individuals are more sensitive to pleasant external stimuli.¹²⁶ Agras et al.¹²⁷ conducted a 2-year prospective study of infant adiposity and feeding patterns. In a cohort of 99 infants, they found that approximately 23% of the variance in BMI at age 2 years was attributable to the frequency of feeding and vigor of sucking style at age 2 weeks. These findings are consonant with the observations that older children¹²⁸ and adults¹²⁹ who are overweight tend to eat more rapidly and chew their food less than lean peers. The existence of different sucking styles at an early age suggests that some aspects of feeding behavior may be inherited.

Energy Expenditure

Though AT represents a significant proportion of body mass (as much as 70% in an obese individual as compared with 15% to 30% in the average nonobese adult), it utilizes relatively little energy (approximately 3% of total resting energy expenditure). LBM includes all energy-consuming tissues (mainly brain, liver, kidney, and muscle), including the small fraction of adipocyte cellular mass that consumes energy. Two individuals of the same body mass but different body composition may differ substantially in resting energy requirements. Accordingly, caloric intake and energy expenditure are better expressed per unit of energy-consuming tissue, i.e., LBM. Adults gain about 0.3 kg of LBM (predominantly muscle) per kg of weight gained in AT. In other words, the excess weight of an obese person is approximately 75% fat and 25% LBM.¹³⁰

Energy expenditure consists of *voluntary* and *obligatory* components. Physical activity accounts for only about 10% of total energy utilization in the average adult.^{131, 132} Obligatory energy expenditure can be subdivided into metabolism, which occurs in response to environmental stimuli (such as the work of digestion or maintenance of body temperature), and the resting metabolic rate (RMR), which reflects primarily the work of cardiac and respiratory muscles, maintaining transmembrane ion gradients, and of protein turnover.

Some investigators have suggested that there is a third compartment to systemic energy expenditure, constituted by metabolic fuel cycles that consume adenosine triphosphate (ATP) without performing useful biochemical work and thereby "waste" the potential energy stored within the chemical bonds of these substrates. Examples include the simultaneous processes of lipolysis and triglyceride synthesis that occur in AT (see section on regulation of lipolysis and lipogenesis), glycolysis-gluconeogenesis in liver, and fatty acid oxidation in brown AT (see section on function of adipose tissue).¹³³⁻¹³⁵ Though the activation of such cycles (perhaps by increased sympathetic nervous system activity) may contribute to the hypermetabolism encountered in severe stress states, such as burns,¹³⁶ their role in normal metabolic homeostasis is, as yet, unclear.¹³⁷ There has been justified skepticism regarding the contribution of hypoactivity of such cycles to obesity in humans.¹³⁸

Studies in both human and animal subjects have demonstrated that total energy expenditure declines and rises more quickly during periods of undernutrition and overnutrition, respectively, than would be predicted from changes in caloric intake or LBM.^{44, 139-143} The changes in energy utilization observed during experimental alterations of body weight suggest that substrate is directed toward or away from metabolic processes of varying efficiency and/or cost depending on body weight and nutrient supply.

The major systemic regulators of the relative activities of these cellular systems in humans are triiodothyronine (T_3) and sympathetic autonomic activity.^{144, 145} Peripheral conversion of thyroxine (T_4) to T_3 increases in response to increased caloric intake and diminishes with weight loss. The resultant changes in metabolic efficiency oppose changes in body weight.¹⁴⁶⁻¹⁴⁹ Catecholaminergic activity relevant to energy homeostasis is more difficult to assess since its major vector is local release of norepinephrine at nerve terminals. Activity at this level may not be well reflected in serum catecholamine levels. However, studies in animals of overall sympathetic nervous system activity (as measured by local catecholamine turnover) have documented a direct correlation with weight change and/or caloric intake.^{144, 150, 151}

There has, to date, been no demonstration of a novel derangement of metabolic efficiency that regularly causes obesity. In fact, mean basal energy requirements (expressed per sq m of body surface area or per unit of LBM) are not significantly different between groups of lean and obese subjects.^{46, 131, 152-155} Though diminished exercise capacity may accompany obesity, lack of physical exercise does not necessarily result in obesity.^{156, 157} A significant number of calories are probably expended in such activities as pacing, fidgeting, etc. However, there are few reliable studies quantifying differences in such activities between lean and obese subjects. Even a small difference in average daily caloric expenditure would, over time, cause significant alterations in body mass if caloric intake were held constant.

Leibel and Hirsch⁴⁶ found that following weight reduction, formerly

obese subjects demonstrated 24-hour weight maintenance energy requirements well below those attributable to decreased body size. Dore et al.¹⁵⁸ detected no difference in RMR between weight-reduced females and normal weight-control subjects. The seeming discrepancy between studies demonstrating a decrease in total energy requirements following weight loss and no change in RMR may reflect changes in activities such as fidgeting, pacing, etc., which are not included in RMR but may be a significant fraction of total energy expenditure. Other studies of energy homeostasis following weight loss have suggested that the high rate of recidivism among the obese may be due to an inability to reduce intake in proportion to the decrease in energy expenditure associated with weight loss.¹³²

Studies of obese adults with a history of familial or juvenile onset obesity have reported decreased thermogenesis following glucose infusion¹⁵⁹⁻¹⁶³ or meal ingestion.^{164, 165} Some investigators have reported that impaired meal- or norepinephrine-induced systemic oxygen consumption do not normalize following weight loss in obese subjects.^{132, 166} The persistence of impaired thermogenesis implies that this defect may be a predisposing factor for obesity rather than a correlative of excess adiposity. However, other investigators have not found such discrepancies in thermogenic response between obese and lean subjects.¹⁶⁷⁻¹⁷¹ The discordance among these reports is probably due more to methodologic than biologic differences. Nonetheless, obesity is clearly a disorder of heterogeneous etiology, and it seems likely that it does, in some instances, reflect subtle interindividual differences in metabolic efficiency.

The relative decrease in energy requirements of the reduced-obese may reflect changes in various energy homeostatic mechanisms acting to return such individuals to a set point for adiposity that is higher in obese than in lean individuals.¹¹⁵ The persistence of a tendency to gain weight and the need to restrict caloric intake even in the long-term reduced obese,⁴⁶ suggest that the set point is not readily changeable. In this sense, obesity may represent a physiologic "correction" for a prior imbalance between energy intake and expenditure.

In summary, the obese individual has necessarily ingested an excess of nutrient energy relative to energy expenditure to reach a state of excess adiposity. It is not clear whether the obesity reflects an increased drive to consume calories (or calorically dense foods) or if a primary decrease in energy expenditure precedes the development of obesity. Obesity is a highly heterogeneous disorder and the relative contributions of energy intake and utilization to its onset and maintenance must be assessed on an individual basis. Certain data regarding the long-term constancy of body fat content and the metabolic characteristics of LH-lesioned animals¹⁷² are consistent with, but not conclusively demonstrative of, a set point for body fat content. Cogent arguments can be made against the relevance of the set point concept to human obesity.¹³⁰ To the extent that it is relevant, this construct assumes that there is some central mechanism of assessing adiposity and that there are outflow tracts from this central "ponderostat" that

influence feeding behavior and/or energy homeostatic mechanisms. "Normal" body composition, in this context, is that at which counterregulatory signals are minimal.

Biochemistry and Development of Adipose Tissue

Obesity may be due to an increase in adipocyte size (hypertrophy) and/or number (hyperplasia).^{173, 174} In the moderately obese patient, adipocyte hypertrophy may be the only morphologic change noted in adipose tissue, but as the severity of obesity increases, so does the likelihood of adipocyte hyperplasia.¹⁷⁵ Hirsch and Batchelor¹⁷⁶ noted that there was little difference in adipocyte volume between the moderately obese (170% to 240% of ideal body weight) and the "superobese" (>240% ideal body weight) and suggested that in humans there may be a maximal adipocyte lipid content of approximately 1.0 $\mu\text{g}/\text{cell}$. Once this cell is reached, any further increase in AT mass must include recruitment of new adipocytes. There are no reports of purely hyperplastic obesity in humans, except for the formerly hypertrophic-hyperplastic obese individual who has lost an amount of adipocyte lipid sufficient to return previously hypertrophic cells to a "normal" volume. Once adipocyte hyperplasia has occurred, it is apparently irreversible. While lipid content of the unilocular adipocyte appears to be highly mutable, and may be normalized by weight loss in obese subjects, there is no clinical or experimental evidence that adipocyte number decreases. Reduced-obese patients who have maintained a normal body weight for many years have just as many adipocytes as they did when they were obese,¹⁷⁴ even though this number may be three times that of never-obese patients.¹⁷⁷

Development of Adipose Tissue

White AT (WAT) is of mesenchymal origin and is first detectable as mature cells in the human fetus at approximately 15 weeks of gestation. Adipocyte size and number continue to increase throughout gestation, achieving maximal growth rates at about the 30th week.¹⁷³ Analysis of the lipid content of adipocytes at different gestational ages^{178, 179} reveals that the ratio of saturated to unsaturated fatty acids of depot fat in the very premature infant (<750 gm) is similar to that in maternal fat stores. During the third trimester, there is a 12-fold increase in total body fat (as compared to a three-fold increase in body weight)¹⁸⁰ and an increase in the relative proportion of saturated free fatty acids. Saturated fatty acids can be of dietary origin or synthesized from carbohydrates in the liver (unlike polyunsaturated fatty acids, which are primarily of dietary origin). Since the composition of circulating maternal fatty acids does not change significantly during the last trimester of pregnancy, these fetal saturated fatty acids arise predominantly as a result of fetal intrahepatic lipogenesis from glucose or

other carbohydrates. This suggests that early gestational adiposity is primarily due to passive transfer of maternal fatty acids, while the bulk of newborn AT is derived from intrafetal carbohydrate metabolism and is, therefore, more regulated by fetal than maternal metabolism. That the size of the carbohydrate load that is available to the fetus may influence fat accretion is apparent in the macrosomic infant of the diabetic mother (see section on environment).

AT accounts for 15% to 30% of the body weight of most adults, though this figure may be substantially reduced in athletes or increased in obese individuals. The percentage body fat of premature or low-birth weight infants may be as low as 3%. Normally, during the last trimester of pregnancy, fetal body fat content increases from 3% to 5% to approximately 12% to 15% of body mass at birth.¹⁷ During the first year of life percent body fat increases to approximately 18% in males and 19% in females, but after age 1 year remains relatively constant until puberty.¹⁸¹⁻¹⁸³ During the pubertal growth spurt, the relative fat content of boys decreases slightly to an average of approximately 17% while the percentage body fat of girls increases to an average of 26%.^{130, 184-187}

Most AT growth during the first 6 months of extrauterine life appears to be via fat cell enlargement rather than hyperplasia. After age 2 years, there is little further increase in adipocyte volume in the nonobese child. It is generally agreed, however, that fat cell number continues to increase slowly until a peripubertal adipose growth spurt occurs.^{188, 189} Knittle et al.¹⁹⁰ performed one-time measures of adipocyte size and number in 178 nonobese and 110 obese children ranging in age from 4 months to 19 years. They obtained follow-up data on 132 of their subjects. They concluded that from 6 months to 1 year of age, increases in fat depot size are predominantly due to increases in adipocyte volume rather than number. They found no significant increase in adipocyte number from ages 2 to 10 years and no significant increase in adipocyte volume from ages 2 to 14 in nonobese subjects. However, in obese subjects, there was continual adipocyte hyperplasia at a time when adipocyte number was relatively stable in nonobese individuals. These data are consistent with the concept (discussed later) that the achievement of a critical adipocyte volume triggers adipocyte hyperplasia. The exact point—relative to puberty—at which adult adipocyte size and number are achieved remains unclear.

While there does appear to be a maximal adipocyte volume, there is no evidence that there is a maximal adipocyte number in humans at any age. The seemingly limitless potential for adipocyte hyperplasia suggests that

1. There may be a pool of cells ("preadipocytes") that are genetically programmed to differentiate into adipocytes under the right conditions. Such cells would be indistinguishable from a mesenchymal cell type unless stimulated to complete differentiation, and/or
2. New adipocytes may be formed throughout life by mitosis of existing

adipocytes. Since the mature adipocyte appears to be incapable of mitosis, the first possibility seems the more likely explanation.¹⁹¹

It is unlikely that preadipocytes are fully differentiated but empty fat cells, since both biochemical and autoradiographic studies of rat fat cells have demonstrated that while very small adipocytes exist, they are not the primary source of apparent "new" cells in hyperplastic adipose tissue.^{191, 192} Several investigators have proposed that preadipocytes arise from pericytes of the capillary endothelium.¹⁹³⁻¹⁹⁵ The observation that newly formed, rapidly growing adipocytes are found near large capillaries, while more mature and stable cells are closest to smaller vessels suggests that patterns of vascular development are somehow related to AT growth.¹⁹²

Regional differences in blood flow to various AT depots may be important in determining rates of lipid deposition in existing adipocytes.¹⁹⁶ The observation in animals and humans that AT blood flow per cell remains constant despite marked changes in cell volume related to age, diet, or body weight supports a possible role of blood supply in the regulation of adipocyte number.¹⁹⁷ This regulation may take the form of substrate supply, removal of lipolytic products, or delivery of metabolic signals regulating adipocyte differentiation and lipid content.^{196, 198, 199} Prinz et al.²⁰⁰ have reported that hyperinsulinemia due to insulin infusion is associated with a decrease in AT blood flow (as is triglyceride infusion or meal ingestion) in rats. Such regulation of blood flow in response to insulin or other signals may serve to regulate adipocyte size and number by "protecting" existing adipocytes and preadipocytes from nutrient excess and mutagenic effects of insulin.

It is not known exactly what factors induce further differentiation of preadipocytes or if there are any means of distinguishing which cells have the potential to differentiate further. Even isolated cloned cell lines of preadipocytes show only a 40% to 90% rate of conversion to mature adipocytes in culture. The factors regulating this *in vitro* differentiation of preadipocytes appear to reflect both the previous and current milieu of the individual cells. Preadipocytes initially appear identical to normal fibroblasts with essentially no detectable lipogenic or lipolytic activity. In culture medium, cells will continue to divide but not differentiate until growth arrest occurs. This is usually caused by confluence of cells, *i.e.*, the complete covering of the culture plate with preadipocytes. Once this has occurred, some of the cells will continue to divide and also to differentiate until colonies of mature adipocytes with a full complement of lipolytic and lipogenic enzymes exist on the plate. These colonies are separated by patches of undifferentiated preadipocytes. If, however, these remaining preadipocytes are given a stimulus to mitose further, *e.g.*, by creating space around them, they will also differentiate.²⁰¹ Both the rate and frequency of preadipocyte conversion to mature adipocytes can be enhanced by incubation with insulin²⁰² and T_3 .²⁰³ Certain classes of prostaglandins seem to inhibit

differentiation of preadipocytes.²⁰⁴ Recent advances in molecular biology and techniques of gene cloning have made it possible to identify many proteins (most as yet uncharacterized) that are sequentially expressed during adipocyte differentiation and that may be involved in the initiation and/or termination of this process.^{205, 206}

Given the seemingly limited potential for adipocyte hypertrophy, some authors have speculated that, in vivo, a signal generated by hypertrophied adipocytes may induce the further differentiation of preadipocytes.^{192, 207, 208} The induction of new adipocytes would serve to reduce the lipid content per cell, with a corresponding reduction in the adipocyte "induction signal."

Function of Adipose Tissue

The major function of AT is to provide stored chemical energy that may be utilized at times of limited food intake. The nonpolar, hydrophobic, and calorically dense triglyceride molecule, which constitutes over 90% of the mass of AT, is well suited to this purpose. The energy content of different tissues are summarized in Table 3. From a structural standpoint, AT also provides thermal insulation and mechanical protection of underlying tissues. Adipocytes also function as storage depots for various steroids and, for example, play an active role in the aromatization of androstenedione to estrone.^{209, 210} The role of AT in steroid storage and metabolism may contribute to the apparent requirement of a minimal AT mass for normal menses and the apparent relationship between adiposity and menarche in females.⁹

In addition to the WAT described earlier, some mammals also have

TABLE 3.
Energy-Storage Sites in a 70-kg Man*

Fuel	Tissue	Grams	kcal
Triglyceride	Adipose	15,000	105,000
Glycogen	Liver	70	280
	Muscle	120	480
Glucose	Body fluids	20	80
Protein	Muscle	6,000	25,000

*From Leibel L, Berry EM, Hirsch J: Biochemistry and development of adipose tissue in man, in Conn HL, DeFelice EA, Kuo P (eds): *Health and Obesity*. New York, Raven Press, 1983. Used by permission.

brown adipose tissue (BAT). The color of this tissue is due to the cytochrome pigments of its densely packed mitochondria. This tissue is located mainly in the mediastinal, intrascapular, axillary, and perirenal regions.²¹¹ Unlike WAT, BAT is probably of neuroectodermal origin²¹² and has a very rich sympathetic nerve and vascular supply.^{213, 214} The major function of BAT appears to be thermogenesis, i.e., the conversion of the chemical energy of the fatty acids of its stored triglycerides directly into heat. This is achieved by the facultative invocation of a mitochondrial pathway that does not permit the formation of high-energy phosphate bonds as ATP.²¹⁵ The uncoupling of BAT mitochondrial substrate oxidation from ATP production appears to be regulated by a 32-kD protein in the inner mitochondrial membrane. This protein, which has been called thermogenin, uncoupling protein, and GDP-binding protein, has been demonstrated in the BAT of animals and human infants, but appears to be absent in WAT, liver, and muscle.^{216–218} In vitro activation of this protein occurs via displacement of a “blocking” purine nucleotide and allows protons to descend an energy gradient across the BAT inner mitochondrial membrane, without “capturing” the resultant energy as ATP.^{215, 219, 220} In addition to BAT’s role in body temperature homeostasis (e.g., arousal from hibernation), it constitutes a potential metabolic buffer against fat accretion secondary to overfeeding. By converting excess calories to heat rather than lipid stores, it could play an active role in body weight regulation.^{140, 221} BAT contributes to nonshivering thermogenesis in the human neonate^{179, 222} and it has been suggested that a defect in BAT may be responsible for some instances of human obesity.^{163, 165, 212, 223} However, there is currently little evidence to support the hypothesis that BAT plays a substantive role in energy homeostasis in the adult human.²²⁴ (This subject is extensively discussed by Trayhurn et al.)²²⁵

Regulation of Lipolysis and Lipogenesis

In WAT, triglycerides are simultaneously synthesized (lipogenesis) and broken down into their constituent free fatty acids and glycerol (lipolysis). The relative activity of these processes determines whether there is a net increase or depletion of lipid within the individual adipocyte, as well as total and regional intraadipocyte lipid stores. The fundamental biochemical processes underlying lipolysis and lipogenesis are shown in Figure 1. A number of points should be emphasized.

1. The rate-limiting step in the storage of dietary fats (i.e., those transported to AT as chylomicrons) is dependent on the activity of the enzyme lipoprotein lipase (LPL). Both chylomicrons and very low-density lipoproteins (VLDL) contain apoprotein C-II, which is a specific activator of LPL.²²⁶ Lipoproteins can also be hydrolyzed by hepatic, cardiac muscle, or skeletal muscle lipases to release free fatty acids. These free fatty acids then circulate bound to albumin and can

enter the adipocyte, probably via a specific free fatty acid carrier protein, and be used in lipogenesis.²²⁷

2. Lipolysis of stored triglycerides is dependent upon the activation of hormone sensitive lipase (HSL). This enzyme is under reciprocal regulation by processes tending to phosphorylate its regulatory site (by activating the adenylate cyclase \rightarrow cAMP \rightarrow protein kinase "cascade") or to dephosphorylate this site. Therefore, agents which favor adenylate cyclase formation, such as β -adrenergic agonists, will increase lipolysis while agents which inhibit cyclase production, such as α -adrenergic agonists and adenosine, are anti-lipolytic. Insulin's anti-lipolytic action may be primarily via its activation of a phosphatase (which dephosphorylates HSL) rather than inhibition of cAMP accumulation.²²⁸
3. There are both lipolytic and antilipolytic pathways that depend upon activation of membrane stimulatory or inhibitory "N" proteins respectively.

Catecholamine-responsive processes may be major means by which body weight and energy expenditure are regulated.^{179, 229-232} Since adipocytes are sensitive to both β_1 - (lipolytic) and α_2 - (antilipolytic) adrenergic stimulation, the relative degree of activation of these opposing systems can

FIG 1.

The biochemistry of lipogenesis and lipolysis. Lipogenesis and lipolysis occur simultaneously. Circulating triglycerides are derived from dietary fats and from fats which are synthesized in the liver. Triglycerides are packaged into lipoproteins that serve as circulating transport micelles. Dietary fats circulate as triglycerides in chylomicron lipoproteins, while triglycerides synthesized in the liver circulate as constituents of very low density lipoproteins (VLDL). Lipoprotein lipase (LPL) hydrolyzes the triglyceride (TG) within these molecules, releasing free fatty acids (FFA) and glycerol. FFA may be returned to the circulation or transported into the adipocyte and esterified to glucose-derived glycerol-phosphate (glycerol-(P)). The triglyceride is then stored in the adipocyte's large lipid storage droplet. Lipolysis depends ultimately upon the activation of intracellular hormone sensitive lipase (see Figure 2). FFA released by lipolysis may be reesterified, or returned to the circulation as albumin-bound FFA. The relative rates of reesterification and FFA release into the circulation depend upon the availability of glucose (for glyceride-glycerol synthesis) as well as local factors such as blood flow. Glycerol that is released from hydrolysis of either lipoprotein or adipocyte triglycerides must be converted to glycerol-phosphate by hepatic glycerol kinase before it can be reutilized in de novo lipogenesis. (Human adipose tissue does not contain functionally significant amounts of glycerol kinase.) The relative rates of lipolysis and lipogenesis determine whether there is net storage or depletion of lipid within an adipocyte. MG = monoglyceride; DG = diglyceride; PPi = inorganic phosphate; CoASH = Coenzyme A. (From Leibel RL, Berry EM, Hirsch J: Biochemistry and development of adipose tissue in man, in Conn HL Jr, DeFelice EA, Kuo P (eds): *Health and Obesity*. New York, Raven Press, 1983. Used by permission.)

direct cellular machinery toward fat storage or lipolysis.^{231, 233, 234} The mechanisms by which these opposing systems operate are illustrated in Figure 2. Epinephrine and norepinephrine are the major regulators of adipocyte α - and β -adrenergic activity. Epinephrine is an adrenal hormone that is released directly into the venous circulation. Norepinephrine is released at nerve terminals and therefore has predominantly local rather than systemic effects.^{144, 235} Epinephrine and norepinephrine are both "mixed" adrenergic agonists and can inhibit or stimulate lipolysis depending upon the tissue's relative responsiveness to α - and β -adrenergic stimuli. Comparisons of the *in vitro* effects of adrenergic agents on lipolysis in AT from different fat depots have suggested that abdominal AT is relatively more responsive to β_1 -activation than gluteal or thigh fat.²³⁶⁻²³⁸ Leibel and Hirsch²³⁹ quantified the relative responsiveness of adipocytes to α - and β -adrenergic stimulation in abdominal and gluteal subcutaneous AT depots in weight-stable obese men and women. They found a relative preponderance of α_2 - (antilipolytic) activity in the gluteal depot and of β_1 - (lipolytic) activity in the abdominal depot in both sexes. Between sexes, women had less α_2 -antilipolytic activity than men in the abdominal fat depot. These data suggest that differences in adrenergic responsiveness may be responsible for the relative ease of weight loss from specific anatomic sites as well as for sex-related differences in AT distribution.

Adenosine and insulin are also important regulators of the relative rates of lipolysis and lipogenesis. Adenosine may exert a tonic antilipolytic effect on adipocytes.^{240, 241} Adenosine is released spontaneously by fat cells (e.g., from catabolism of cAMP) and may be part of a "short-loop" regulatory system at the adipocyte level. Insulin stimulates lipogenesis by inducing LPL and enhancing the uptake of glucose into the adipocyte and its conversion to glycerol. Insulin also diminishes lipolysis by inhibiting HSL.

The adipocytes of children and adults respond differently to *in vitro* lipogenic and lipolytic stimuli. Taniguchi et al.²⁴² studied basal and insulin-stimulated conversion of glucose to lipid in adipocytes of different sizes from 20 subjects aged 3 months to 67 years. The basal and insulin-stimulated rates of glucose to lipid conversion were higher in subjects who were still growing, even when corrected for differences due to variability of fat cell size. Marcus et al.²⁴³ measured *in vitro* rates of catecholamine-stimulated lipolysis and antilipolysis in adipose tissue from 36 infants and 12 adults. α_2 -Adrenergic stimulated antilipolysis was significantly greater in infants than adults. On the basis of these two studies, it appears that the AT of infants and children is more responsive to lipogenic and antilipolytic stimuli than that of adults. This combination of metabolic characteristics would tend to favor fat accretion in the child.

Despite our increasing understanding of the controls of lipogenesis and lipolysis in AT, the reader should not lose sight of the fact that net systemic energy balance, and not local regulation of adipocyte metabolism, dictates whether there will be a net change in AT mass.

Role in Energy Homeostasis

As emphasized earlier, the quantity of lipid stored in AT at a specific moment in time represents an integrated reflection of net energy balance up to that point. A critical issue with regard to the physiology of the regulation of lipid stores is whether AT itself somehow participates in the control of its own mass. In analogy with electrical circuitry, AT may simply be “in parallel” with systems subserving the control of energy intake and output. That is, AT mass may be a passive reflection of these processes and not participate in their regulation. Alternatively, AT (or some correlative such as adipocyte volume) may be “in series” with these control systems, somehow interacting with them to modulate energy intake and/or output.

The mechanisms by which such feedback from AT might occur remain unclear. Adipocytes might actively generate signals in the form of hormones or might influence the plasma concentration of other potential messengers such as glucose, insulin, glycerol, and/or free fatty acids.²⁴⁴ Such a “lipostatic system” would provide a means of regulating body weight by providing information to central systems regarding the time-integrated nutritional status of the organism (as reflected in fat stores). These systems (e.g., in brain and/or liver) might then act accordingly to alter feeding behavior or metabolic efficiency to maintain a specific adipocyte volume.^{179, 223, 245, 246} As discussed earlier, there appears to be a maximal adipocyte volume that, once attained, either stimulates or disinhibits the recruitment and further differentiation of preadipocytes. Some studies in rodents suggest that adipocyte volume is “sensed” by the organism and modulates both feeding behavior and energy efficiency.^{192, 207, 247–249} These signals might be greater in the hypercellular obese animal because there are more fat cells generating putative signals. The observation in humans that obese individuals experience the greatest difficulty in adhering to dietary restrictions once a “subnormal” adipocyte volume is reached²⁵⁰ provides indirect evidence that adipocyte volume may influence feeding behavior and/or metabolic efficiency in humans.

In summary, the long-term constancy of body weight and the changes in energy expenditure and feeding behavior observed following weight reduction in humans suggest that body weight is somehow regulated. While specific disorders of either energy intake or expenditure can be implicated in various rodent models of obesity, the problem in humans appears to be much more complex. Human obesity undoubtedly represents a subtle interplay of genetic and environmental determinants of body weight and energy homeostatic mechanisms.

Morbidity

While some of morbidities related to adiposity may be apparent in childhood, the majority are not evident until later in life. It remains unclear

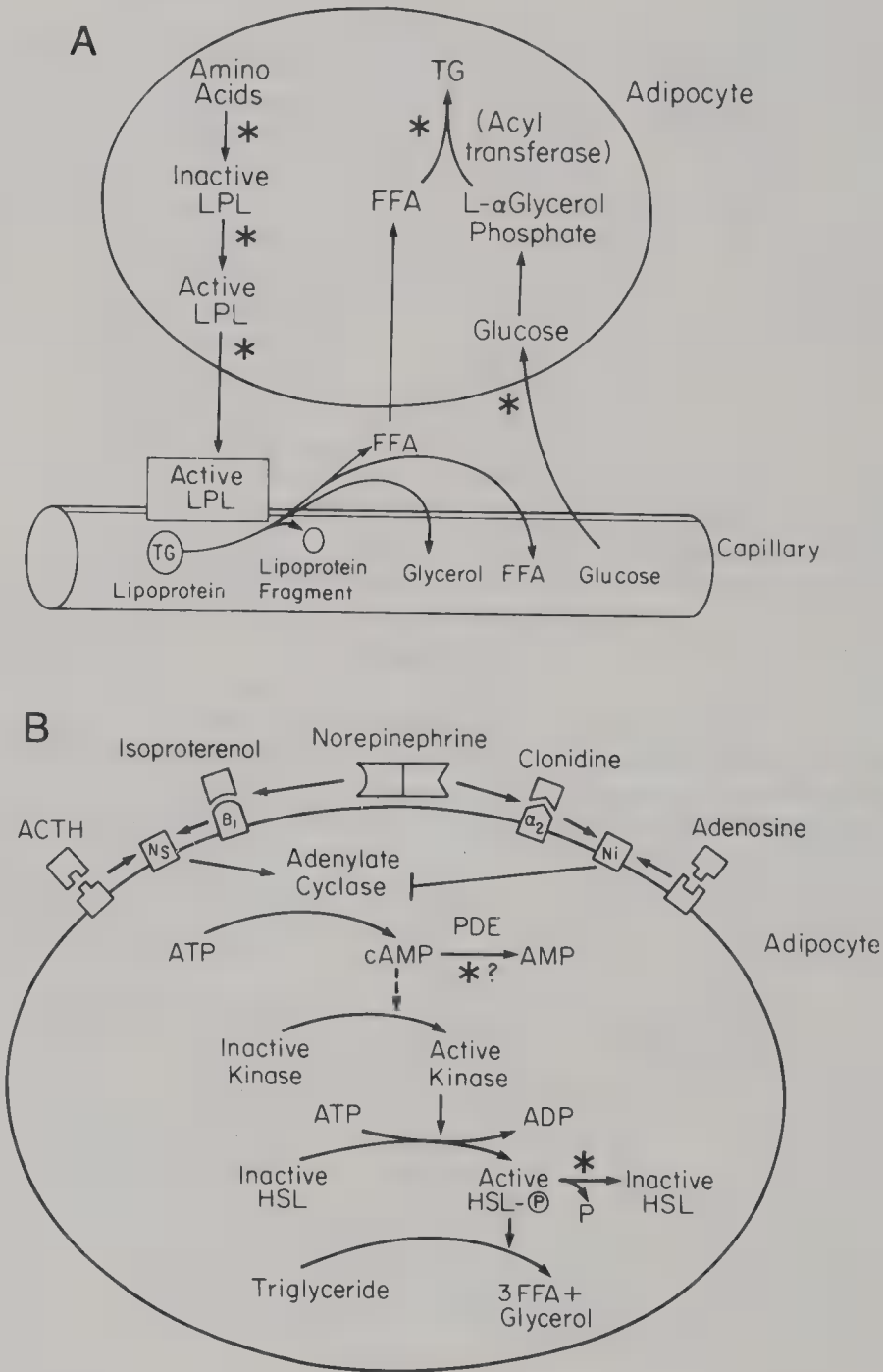


FIG 2.

Activation of lipogenesis (A) and lipolysis (B). The enzyme lipoprotein lipase (LPL) hydrolyzes triglycerides (TG) which are constituents of lipoproteins to free fatty acids (FFA) and glycerol (molar ratio FFA:glycerol = 3:1). The FFA can then be returned to the circulation or transported into the adipocyte to be esterified and stored as triglyceride. Insulin stimulates almost every step in the process of lipo-

whether obesity in childhood constitutes an independent risk factor for adiposity-related morbidity in adulthood, i.e., if morbidity is higher among adults who were obese children even if the obesity does not persist. A discussion of the morbidity associated with pediatric obesity must include both the immediate and long-term sequelae.

Psychologic Morbidities

There are few objective studies of the psychologic sequelae of pediatric obesity. Stunkard et al.^{251, 252} reported that body image disturbances, characterized by feelings of being loathsome and of being viewed contemptuously by others, are significantly more frequent in obese than non-obese adolescents. Though obese preadolescents showed symptoms of the same psychiatric disturbances, these symptoms were less severe than in subjects who had become obese as adolescents or in those whose preadolescent obesity persisted into adolescence. Others²⁵³⁻²⁵⁵ have also found that depression and poor self-esteem are significantly more prevalent in obese children than in their lean peers. It should be noted, however, that other investigators have not found any differences in ratings of self-esteem or body image between obese or lean children.²⁵⁵⁻²⁵⁷ In fact, Stewart and Brook²⁵⁸ found that obese adults were significantly less anxious and depressed than normal-weight persons. Thus, individuals dealing with obese children should avoid jumping to conclusions regarding the psychologic impact of obesity in any specific instance.

Though there is some debate in the literature as to whether there is an increased incidence of personality disturbance in the obese child, there is no doubt that the obese child is subjected to considerable psychologic stress. Many members of modern western societies view obesity as a consequence of self-indulgence and overgratification. The obese child is gen-

genesis. Lipolysis depends upon the activation of an adenylate cyclase-dependent metabolic "cascade" that culminates in the phosphorylation of intracellular hormone sensitive lipase (HSL). HSL hydrolyzes triglycerides to FFA and glycerol. Certain molecules favor or inhibit lipolysis by increasing or decreasing the activity of adenylate cyclase. Such stimulation or inhibition occurs via binding of specific molecules to adipocyte membrane receptors. The activated receptor complex then binds to membrane stimulatory (N_s) or inhibitory (N_i) proteins. The N_s protein stimulates adenylate cyclase and thereby activates the lipolytic cascade. The N_i protein inhibits adenylate cyclase and thus decrease the rate of lipolysis. Norepinephrine and epinephrine are "mixed" agonists, and simultaneously activate both β_1 (lipolytic) and α_2 (antilipolytic) receptors. ACTH has been shown to promote lipolysis in rodent adipocytes but not, as yet, in humans. Adenosine is released by adipocytes (as a product of cAMP metabolism) into the pericellular space, and probably acts as a tonic inhibitor of lipolysis. Insulin-induced antilipolysis is apparently mediated by effects distal to adenylate cyclase. * = insulin effect; PDE = phosphodiesterase; cAMP = cyclic AMP.

erally portrayed in the media as an unattractive, clumsy glutton. Conversely, these same societies laud extreme thinness as an ideal.²⁵⁹ Preadolescent children tend to tease and humiliate the obese child while adolescents isolate their obese peers from parties and other social rites of passage that are important to normal development of peer group and male-female relations. Richardson et al.²⁶⁰ asked children to rate individuals as potential playmates based on questionnaires and photographs and reported that a child will choose a playmate with a major physical handicap or in a wheelchair over one who is obese. Lerner and Gellert²⁶¹ reported that even kindergarteners demonstrate negative attitudes toward their obese peers. Pugliese et al.²⁶² described growth failure and delayed puberty in 14 children aged 9 to 17 years, none of whom were classified as anorectic, due to self-imposed caloric restriction arising from fears of becoming obese.

There is a significant difference in the prevalence of adiposity-related personality disorders between adolescent males and females, despite the fact that the prevalence of obesity based on BMI is approximately 25% among both sexes. Adolescent girls are particularly vulnerable because of their self-consciousness regarding physical development. Huenemann et al.²⁶³ reported that 50% of high school girls felt that they were overweight and 65% expressed a desire to lose weight. Boys, on the other hand, were generally satisfied with their weight or wanted to gain weight.²⁶⁴

Investigators have endeavored to identify specific characteristics of obese individuals that correlate with the likelihood of associated psychologic derangements. Retrospective studies of obese adults have indicated that young women of middle or upper socioeconomic status who have been obese since childhood appear to be the group most likely to suffer disturbances of body image.²⁵² The observation that the presence of obesity-related psychologic disturbances in adulthood is generally limited to those with juvenile-onset obesity implies that childhood is the period of greatest risk for the development of adverse psychologic sequelae of obesity.

Medical Morbidities

Obesity adversely affects many organ systems in adults and children. The relative risk of hypertension among obese adults (BMI >27.8 for men, 27.3 for women) aged 20 to 45 years is 5.6 times that of their nonobese counterparts. In the same age group, the risks of diabetes and of hypercholesterolemia (>250 mg/dl fasting) are increased 2.9 and 1.5 times, respectively.^{265, 266} It has been demonstrated that insulin promotes sodium reabsorption in the distal renal tubule.²⁶⁷ Some authors have suggested that adiposity-related hyperinsulinemia may also promote hypertension by increasing renal sodium retention, which may then result in an increased intravascular volume.²⁶⁸ Other investigators have hypothesized the adiposity-

ity-related hypertension is due to increased sympathetic nervous system activity.²⁶⁹ James et al.²⁷⁰ found that normotensive obese women decreased their excretion of urinary catecholamine metabolites during weight loss and increased on refeeding. In obese women, however, the decrease in urinary catecholamine metabolites during weight loss was not as pronounced as in lean women, suggesting that obesity may represent a primary abnormality of sympathetic nervous system "responsivity" to internal metabolic cues.

These risk factors for cardiovascular morbidity are further exacerbated by the increased cardiovascular demand imposed by obesity.^{271–273} Large studies of obesity-related mortality^{274–279} found significantly higher mortality ratios among overweight men and women (defined as 40% above average weight for age and sex) for cardiovascular disease, stroke, cancer, and digestive disease. Investigators conducting the Framingham study found that the overall mortality rate of nonsmoking, overweight (body weight >10% above average weight for age and sex) males, aged 30 to 49 years and clinically free of cardiovascular disease at enrollment, was 3.9 times higher than in nonsmoking lean males over a 26-year study period.^{274, 278} Obese adults have a higher incidence of osteoarthritis and gout,^{280–282} and are at risk for other dermatologic and orthopedic problems discussed later.

There is also significant pediatric adiposity-related morbidity. Overweight infants suffer a higher incidence of respiratory infections.^{283–286} Pulmonary function tests reveal ventilation/perfusion abnormalities that decrease arterial oxygenation.^{287–290} Abnormalities of respiratory muscle function^{291, 292} and central respiratory regulation²⁹³ can culminate in the Pickwickian syndrome in children or adults.

Obesity represents the most common cause of hypertension in children. Court et al.²⁹⁴ reported that of 309 children who were classified as obese by triceps skinfold thickness >85th percentile, 114 were hypertensive (systolic and/or diastolic blood pressure >90th percentile). Other risk factors for cardiovascular disease, including hypercholesterolemia, elevated low-density lipoproteins, and decreased high-density lipoproteins, correlate positively with excess adiposity. In the Muscatine study, among those in the highest decile for weight, 17.7%, 24.3%, 28.4%, and 28.6% were also in the top decile for plasma concentrations of cholesterol and triglycerides, diastolic blood pressure, and systolic blood pressure, respectively.^{274, 295–297}

Obese children and adults are prone to a variety of dermatologic and orthopedic problems. Overlapping skinfolds are common sites for intertrigo and furunculosis. Obesity is the most common cause of acanthosis nigricans, a condition characterized by papillomatosis, hyperkeratosis, and hyperpigmentation of the epidermis. The etiology of this disorder remains unclear. It is clearly associated with hyperinsulinism and hyperandrogenism (HAIR-AN syndrome) and resolves with weight loss.^{298, 299} Coxa vara,

slipped femoral epiphyses, Blount's disease, and Legg-Calve-Perthé's disease occur with highest frequency in the obese adolescent.³⁰⁰⁻³⁰²

Immunologic function has been repeatedly studied in obese children. Chandra and Kutty³⁰³ found that while numbers of T- and B-lymphocytes, the C3 and C4 components of complement, and serum immunoglobulins were all normal in obese adolescents, 38% of the subjects showed an impairment of cell-mediated immunity and polymorphonuclear leukocyte killing capacity. Other studies have reported a decrease in lymphocyte generation of migration inhibiting factor and maturation rates of monocytes into macrophages.^{304, 305} A number of these obese subjects were subsequently found to have subclinical copper and/or zinc deficiency, and their immunologic abnormalities resolved once these trace metal deficiencies were corrected.

It is difficult to ascertain whether those morbidities traditionally attributed to obesity, such as hypertension, are in fact secondary to excess adiposity or merely covariant expressions of underlying predispositions to both disorders. In addition to possible genetic and physiologic explanations for such concordance, Larsson et al.³⁰⁶ have recently suggested that the android AT distribution and its "sequelae" are due to both aberrant responses to environmental stress that result in relative hypercortisolism. Some authors have suggested that the normotensive, nondiabetic, moderately obese adult is somehow protected by elevated serum estrogen levels, stress adaptation, or some other obesity-related factor from cardiovascular disease and other morbidities usually attributed to obesity.^{277, 307, 308} However, these studies generally fail to consider important covariants of leanness, such as chronic disease and cigarette smoking, which raise overall mortality rates of the nonobese.

Risk Factors for Adiposity-Related Morbidity

Numerous investigators have attempted to quantify the extent to which a history of childhood or early adult obesity correlates with the incidence of morbidity in later adulthood. Tracking studies of obesity, hypertension, and serum lipid values from childhood to adulthood have indicated that these variables track well, i.e., that the obese hypertensive hypercholesterolemic child is likely to remain so over many years.^{274, 309, 310} The observation that hypercholesterolemia, obesity, and other factors associated with an increased risk of subsequent cardiovascular disease that are noted in childhood tend to persist into adulthood emphasizes the importance of routine screening of these variables in children to identify those at risk for subsequent morbidity. In the Framingham study, a strong positive correlation was found between relative weight of adults at entrance into the study and death occurring in any subsequent time interval, independent of interim weight changes.³¹¹ These data suggest that the adverse affects of obesity are not wholly reversible or that genetic influences on this outcome

may cosegregate with genes affecting body weight and may be expressed even if obesity is no longer present. Alternatively, obesity, or obesity-related metabolic changes, at any time in life may constitute causal elements in subsequent morbidity even if the obesity does not persist.

Another important issue is what factors, if any, in the seemingly healthy obese subject may be predictive of later morbidity. Age is clearly important in assessing the relative risk of adiposity-related morbidity. The prevalence of hypertension among individuals with BMI above the 85th percentile is 2.1, 5.6, and 2.9 times as great as in corresponding nonobese populations at age 20 to 29, 20 to 44, and 45 to 74 years, respectively. The prevalence of hypercholesterolemia among overweight adults aged 20 to 44 years is 2.1 times that of their nonoverweight peers, while there is no difference in the prevalence of hypercholesterolemia between obese and nonobese 45- to 74-year-olds.²⁶⁵ Though these studies failed to distinguish subjects on the basis of duration of obesity, they suggest that the older obese individual is at a lower risk of adiposity-related morbidity than the younger obese. Alternatively, obese people who have survived past the age of 45 may be a population selected for their "hardiness."

Adipose Tissue Distribution

In 1956, Vague³¹² first suggested that AT distribution may be better correlated than absolute adiposity with the risk of current or subsequent morbidity. Since then, a substantial body of epidemiologic and physiologic data has supported this hypothesis.^{313, 314} The predominantly truncal distribution of adiposity (android pattern, abdominal/gluteal circumference ratio >0.90 in females, >1.0 in males) is associated with a higher risk of stroke, ischemic heart disease, and overall mortality than the gynecoid pattern (abdominal/gluteal circumference ratio <0.75 in females, <0.85 in males) of fat distribution. An android individual whose BMI is in the lowest population tercile appears to be at a greater risk of adiposity-related morbidity than a gynecoid individual whose weight for height is in the upper population tercile.³¹⁴⁻³¹⁷ These distinctions are based mainly on prospective studies of relatively small groups of subjects in Sweden who were divided into terciles or quintiles based on BMI, and the abdominal/gluteal circumference ratio, and then followed for the incidence of various morbidities. Larsson et al.³¹⁵ studied 792 men, all of whom were 54 years old at entry into the study, over a 13-year period. The incidence of ischemic heart disease and of overall mortality was highest in those in the lowest tercile for BMI but the highest tercile for waist to hip ratio, while the risk of stroke was highest among men in the highest tercile for both of these indices. In a study of 1,462 women³¹⁶ (38 to 60 years old at the time of entry into the study), the 12-year incidence of myocardial infarction, stroke, angina pectoris, and overall mortality showed a significant positive correlation with the ratio of waist to hip circumference. The association with myocardial infarction was independent of age, BMI, serum

cholesterol and triglycerides, and systolic blood pressure. Others^{314, 318} reported significant positive correlations of waist to hip ratio and blood pressure and integrated plasma glucose and insulin responses during an oral glucose tolerance test in obese and nonobese premenopausal women.

In adults, subscapular skinfold thickness correlates well with abdominal circumference while the triceps skinfold correlates with pelvic circumference.⁴⁰ While such comparisons have not, as yet, been reported in children, studies of skinfold thicknesses in children have suggested associations of adipose tissue distribution with morbidity that are similar to those seen in adults. Blood pressure varies directly with subscapular skinfold thickness in children and adults.^{28, 33, 35-38} Dahlstrom et al.³⁷ examined skinfold thicknesses, weight, height, and circulating levels of lipoproteins, cholesterol, and insulin in 3,596 Finnish children aged 3 to 18. Subscapular skinfold thickness correlated significantly with serum insulin levels in boys and girls over 6 years of age. Correlations between insulin and triceps skinfold thickness were not as strong. The observation that subscapular skinfold thickness correlates highly with both morbidity (blood pressure and cholesterol) and abdominal circumference (android fat distribution) further emphasizes the importance of assessing fat distribution as well as absolute fatness.

Retrospective and prospective studies of childhood and adult adiposity indicate that the older an obese child, the greater is the likelihood that the obesity will persist into adulthood. Such tracking is independent of duration of obesity. The obese adolescent who has been obese for 2 years is still at greater risk of adult obesity than the obese 10-year-old who has been obese for 8 years.^{1, 319, 320} Charney et al.³²¹ showed that the obese infant (less than 2 years old) was 2.33 times more likely to become an obese adult. This relative risk increases to 6.3 among adolescent boys.^{265, 319, 322} Prospective studies of skinfold thicknesses have demonstrated that the correlation of triceps and subscapular skinfold thickness percentiles in childhood and adulthood increase steadily with age. Therefore, the adolescent whose subscapular skinfold thickness is above the 90th percentile is much more likely to become an adult with excessive truncal fat than the infant with a similar subscapular skinfold thickness. In all age groups, however, the greater the percentile rank of triceps or subscapular skinfold thickness, the more likely the child is to remain at that rank into adulthood.²⁷⁷

Endocrinology of Pediatric Obesity

Though many aspects of the endocrine system are affected by obesity, in virtually all instances these changes are covariants of body weight or caloric intake that normalize with weight reduction. In other words, *these endocrine changes are generally not causal agents of obesity.*

Hypothalamic-Pituitary Axis

Basal serum growth hormone (GH) concentrations, as well as GH release in response to hypothalamic stimuli (clonidine, hypoglycemia, etc.) and direct somatotroph stimulation (growth hormone releasing hormone, GRH), are diminished in obese children and adults.^{218, 323-329} Nonetheless, obese children demonstrate accelerated linear growth and advanced bone age and maintain normal sensitivity to the lipolytic and calorigenic actions of growth hormone.³³⁰⁻³³² Similarly, normal subjects who are overfed until they become obese demonstrate diminished basal and stimulated growth hormone release.^{44, 329, 333} The impaired secretion of growth hormone in obesity is clearly related to AT excess rather than body size, since the effect is fully reversible with weight loss, and subjects of comparable weight due to increased muscle mass, such as weight lifters, have normal GH secretory patterns.³³⁴⁻³³⁷

The mechanisms underlying the diminished GH secretion of obesity remain unclear. Obese individuals do not have an absolute deficiency of GH, since responses to provocative stimuli can be further augmented by β -receptor blockade or prior administration of T_3 .^{338, 339} It has been suggested that the elevated free fatty acid levels of obese subjects may inhibit pituitary response to GRH.³⁴⁰⁻³⁴⁴ However, reduction of circulating free fatty acids by administration of nicotinic acid (an antilipolytic agent) fails to return basal serum GH concentrations to normal in the obese.³⁴⁵⁻³⁴⁹

The major mechanism by which GH promotes somatic growth is by the stimulation of the synthesis of somatomedins (insulin-like growth factors, ICFs), which are produced mainly in the liver. Serum concentrations of somatomedins in obese children are generally normal or elevated, despite concurrent low concentrations of plasma GH.³⁵⁰⁻³⁵² Hepatic synthesis of somatomedins is stimulated by insulin as well as GH perfusion,³⁵⁰ and malnourished subjects, who have low serum concentrations of insulin but high GH concentrations, also tend to have diminished serum concentrations of somatomedins. The preceding observations suggest that somatomedin production is more dependent upon insulin than GH.³⁵³ The hyperinsulinemia associated with obesity may contribute to the supranormal statural growth velocities often seen in the obese child. The negative feedback of insulin-provoked somatomedin on the pituitary somatotrophs may explain the diminished serum concentration of GH seen in the obese.³⁵⁴⁻³⁵⁷ Additionally, some authors have speculated that the binding of somatomedin to its plasma transport protein may be decreased in the obese state, creating a larger bioactive pool to promote somatotroph suppression and/or somatic growth.³²⁹ Alternatively, insulin itself, which has been shown to decrease somatotroph responsiveness to GH releasing factor in vitro, may be the mediator of suppressed GH release in obesity.^{358, 359}

GH has an in vitro lipolytic effect on adipocytes that is independent of its effect on glucose transport.^{332, 360} GH-deficient children usually have an

android distribution of fat, i.e., that distribution of fat associated with the highest morbidity in adults.³¹⁴ The adiposity-related diminution of serum GH concentration may thus exacerbate the obese state by promoting a more morbid distribution of adipose tissue.

Prolactin production is also altered in the obese child. Though basal serum prolactin concentrations tend to be elevated in these subjects, lactotroph responses to insulin-induced hypoglycemia and thyrotropin-releasing hormone are significantly diminished.³⁶¹⁻³⁶⁴ The physiologic mechanisms underlying these changes in lactotroph response are not known.³⁶⁵ Kolesnick et al.³⁶⁶ reported that polyunsaturated free fatty acids enhanced prolactin release from rat lactotrophs in vitro, suggesting that the increase in circulating free fatty acids associated with obesity may also play a role in the elevation of prolactin concentrations.

Adrenal

Studies of rodents with autosomal recessive genetic obesity, such as the ob/ob mouse or Zucker rat, suggest that adrenal steroids play an important permissive role in the phenotypic expression of the obese genotype. Phenotypic expression of the obese genotype is impaired, for example, in the adrenalectomized ob/ob mouse. While adrenalectomized animals do have a higher percentage of body fat than homozygous normal controls, they do not become as obese as their nonadrenalectomized peers until excess glucocorticoids are provided.^{47, 124} Glucocorticoids are potent appetite stimulants in humans and animals. They may affect expression of an underlying obese genotype by stimulating or disinhibiting feeding. However, even with pair-feeding, adrenalectomized animals fail to gain weight at a rate similar to their sham-operated littermates, suggesting that glucocorticoids also affect energy utilization in these genetically obese rodents.

Glucocorticoid production is increased in obese children,³⁶² reflecting increased LBM, an enlarged intraadipocyte space for steroid storage, and changes in adrenal steroid metabolism. Plasma cortisol (free and bound), circadian rhythms of cortisol secretion, and urinary free cortisol are all normal in obese subjects, while there appears to be a slight increase in 24-hour integrated serum ACTH concentration.^{362, 367, 368} The urinary excretion of 17-hydroxysteroids, which are glucocorticoid metabolites, is normal when corrected for the increased LBM that accompanies obesity (see previous section on energy intake).³⁶⁹ The increase in the net excretion of urinary glucocorticoid metabolites, in the context of normal serum cortisol concentrations, suggests that cortisol turnover is increased.

The increase in ACTH release also increases sex steroid production by the adrenal zona reticularis. Unlike the glucocorticoids, sex steroid production appears to be increased to a greater extent than sex steroid clearance. Serum concentrations of adrenal androgens are higher in obese children than in their age-matched or Tanner stage-matched lean peers even though urinary excretion of 17-ketosteroids (androgen metabolites) is in-

creased in obese children and adults when corrected for LBM.³⁷⁰ The abnormalities in adrenal sex steroid metabolism noted in obese children may be largely attributable to increased cortisol turnover. The increased rate of cortisol clearance in the obese results in diminished negative feedback on pituitary ACTH release. The resulting increase in ACTH stimulates the adrenal zona fasciculata until normal cortisol levels are attained. Increased ACTH also drives the adrenal zona reticularis to produce more sex steroids. Since sex steroid clearance is not increased sufficiently in obese individuals to accommodate this increased production, there is a net increase in circulating adrenal sex steroid concentrations. This phenomenon may explain the earlier adrenarche often seen in obese children. By 7 to 9 years of age, obese girls may have circulating adrenal androgen concentrations equal to those of normal adults. Prepubertal obese boys also demonstrate an increase in adrenal androgen production that plateaus in early adolescence.³⁷¹

Dehydroepiandrosterone (DHEA) is an adrenal steroid that serves as a precursor of adrenal sex steroids. DHEA can be converted in the adrenal gland to a weak androgen, DHEA-sulfate, or to etiocholanolones that have no sex steroid bioactivity. DHEA metabolism in obese subjects is of special interest because administration of exogenous DHEA to animals is accompanied by a marked diminution in rates of fat accretion, i.e., an antiobesity effect.^{372, 373} Genetically obese ob/ob mice and Zucker rats gain weight at rates comparable to lean controls during DHEA administration, but rapidly become obese when DHEA is discontinued. This effect is not due to diminished food intake and, unlike hypocaloric diets or appetite suppressant regimens, is not associated with growth retardation of any other organ system. Comparisons of AT cellularity and cell size in different fat depots in DHEA-fed versus control Zucker fatty rats have revealed interdepot differences in the inhibition of fat accretion during DHEA administration.^{372, 374} This observation suggests that DHEA influences fat distribution as well as its rate of accretion. As discussed in the section on AT distribution, the anatomic distribution of AT in humans is related to current and subsequent morbidity.

Obese human subjects have elevated serum concentrations of DHEA. These could reflect changes in DHEA synthesis, storage, or clearance. There appears to be an increased intraadipocyte storage pool of DHEA in obese subjects. Interestingly, these same obese subjects demonstrate a decrease in intraadipocyte storage of DHEA-sulfate. These data suggest that there is an increased uptake of DHEA by AT in the obese and a decrease in peripheral conversion of DHEA to its sulfated metabolite.³⁷⁵⁻³⁷⁷ The mechanisms underlying the antiobesity effects of DHEA remain unclear. The antiobesity effects of DHEA in rats can be mimicked by administration of DHEA-sulfate or etiocholanolone (the DHEA metabolites without androgenic activity), but not by administration of androstenedione or other sex steroids. As discussed earlier, corticosteroids appear to play a permissive role in the expression of the obese genotype in various animal models

of obesity and are also potent stimulants of appetite. Theoretically, DHEA may somehow alter corticosteroid responsiveness of either energy intake or output systems, though it has been noted that the antiobesity effects of DHEA are independent of food intake. Alternatively, DHEA may increase energy output by promoting activity of some energy-costly metabolic process. While various possible energy-wasting pathways have been proposed as the site of DHEA action, stimulation of these proposed pathways with agents other than DHEA has failed to mimic the antiobesity effects of DHEA.³⁷⁸⁻³⁸⁰ Further work is clearly needed to elucidate DHEA's role in energy homeostasis and its potential use, if any, in the treatment of obesity.

Thyroid

Because obesity may be a manifestation of hypothyroidism, and because of the central role of thyroid hormone in energy homeostasis (see previous section on energy expenditure), thyroid hormones have been extensively evaluated in the obese. Obese children have mildly elevated serum concentrations of T_3 , probably reflecting an increased rate of peripheral synthesis from T_4 . Baseline T_4 and reverse T_3 concentrations are normal, but T_4 release in response to thyroid-stimulating hormone (TSH) is significantly diminished.³⁸¹ Bray et al.³⁸² hypothesize that there is some obesity-related factor that stimulates the thyroid gland while inhibiting its responsiveness to TSH. Obese subjects may also be somewhat resistant to the actions of thyroid hormone.³⁸³ This suggestion is supported by the finding that obese subjects appear to have decreased concentrations of T_3 receptors in solubilized nuclei of circulating monocytes,³⁸⁴ and prolonged recovery of the Achilles tendon reflex.^{385, 386} All of these changes are fully reversible with weight loss and can be mimicked by overfeeding the normal-weight subject.^{146, 147}

Endocrine Pancreas

As discussed in the section on energy intake, the pancreas appears to play a pivotal role in systems of energy homeostasis.¹²⁴ Circulating glucose, insulin, and ketone levels may influence hypothalamic feeding and satiety systems. Vagal efferents from the hypothalamus stimulate insulin and glucagon release from the pancreas.^{95, 387} In certain rodent models of obesity, such as the Zucker rat, hyperinsulinemia appears to be a major factor in the recruitment of new fat cells.^{388, 389} Sympathetic nervous inputs also influence pancreatic insulin release. α -Adrenergic stimulation diminishes insulin release, while β -adrenergic activation tends to increase insulin release.^{390, 391}

Hyperinsulinemia and insulin resistance are well-known concomitants of obesity. The oral glucose tolerance test in the obese individual typically demonstrates peripheral resistance to insulin as manifested by an increase

in the insulin/glucose ratio and in the amount of insulin required to maintain normal glucose levels.³⁹² Obesity is the most common cause of insulin resistance in adults and children.³⁹³ However, chemical diabetes secondary to obesity is rare in children.^{394, 395} Responsiveness of AT to insulin-stimulated glucose uptake in vitro is inversely correlated with adipocyte size.³⁹⁶ Serum immunoreactive insulin levels and fat cell diameter are directly correlated in obese adults and in lean subjects with enlarged adipocytes secondary to type IV hyperlipidemia.^{397–399} In contrast, obese subjects are not resistant to insulin action on noncarbohydrate systems such as amino acid metabolism, activation of lipoprotein lipase, and antilipolysis.^{400–402} The hyperinsulinemia of obesity thus favors a further increase in lipid stores by inhibiting lipolysis and stimulating lipogenesis via activation of lipoprotein lipase (see section on regulation of lipolysis and lipogenesis).

The mechanisms of adiposity-related insulin resistance involve receptor and postreceptor modifications that are not limited to AT. Hyperinsulinemic obese subjects demonstrate decreases in insulin receptor binding in hepatocytes, monocytes, and myocytes as well as adipocytes. The ubiquitous nature of these insulin receptor changes suggests that there may be homologous regulation of insulin binding to many tissues. Alternatively, the hyperinsulinemia of obesity (in response to adipocyte hypertrophy) may lead to a secondary downregulation of insulin receptor binding in other tissues.⁴⁰³ However, a decrease in receptor binding is not sufficient to explain the changes in the in vitro cellular glucose transport/insulin dose-response curves seen in obese subjects. Adipocytes and muscle cells have many “spare” insulin receptors. It is necessary to occupy only a small fraction of the total number of insulin receptors in order to achieve maximal biologic effect on glucose transport.^{404–407} Large fat cells demonstrate both a decrease in the number of insulin receptors (both per cell and per unit cell membrane area)⁴⁰⁸ manifested as a right shift of the dose-response curve, and a decrease in responsiveness (maximum level) of insulin-stimulated rates of glucose transport.^{409–411} The coupling of the insulin-receptor complex to the glucose transport system appears to be normal in large fat cells, i.e., once the insulin-receptor complex has formed it efficiently initiates glucose transport.^{403, 412} It is most likely, therefore, that there is a postreceptor modification of insulin-mediated glucose transport in the enlarged adipocyte as well as a decrease in the number or affinity of the insulin receptors.

Gonads

The plasma concentrations of testosterone in obese prepubertal boys are comparable to those of Tanner stage-matched lean controls. However, testicular responsiveness to human chorionic gonadotropin and to gonadotropin-releasing-hormone–provoked gonadotropin release appears to be diminished in obese boys.³⁷¹ The apparent gonadal hyporesponsiveness may be an artifact of an adiposity-related reduction in sex hormone bind-

ing globulin, which results in a normal bioactive pool despite diminished poststimulation total serum concentrations of circulating sex steroids in the obese.⁴¹³ Gross obesity in women is associated with amenorrhea, dysfunctional uterine bleeding, polycystic ovary syndrome, and inadequate maturation of the uterine stroma during the luteal phase of the menstrual cycle.^{329, 414} There are numerous possible adiposity-related changes in the production of adrenal or gonadal sex steroids that may account for these menstrual irregularities. In addition to the premature adrenarche discussed in the section on adrenal steroids, *in vitro* studies of ovarian stroma have demonstrated that high ambient concentrations of insulin are associated with an increase in ovarian androgen production. Alternatively, there may be an overproduction of estrogen due to aromatization of circulating androstenedione to estrone by the enlarged adipocyte pool.^{209, 415, 416} Adiposity-related menstrual abnormalities, with the exception of polycystic ovary syndrome, similar to most of the endocrinopathies discussed earlier, are usually reversible with weight loss.

In summary, there are adiposity-related endocrinopathies involving both central and peripheral endocrine systems. Almost all of these will resolve with weight loss. Physicians must bear in mind that many seemingly abnormal endocrine tests will be secondary to obesity rather than indicative of primary endocrine pathology.

The Relative Contributions of Genotype and Environment to Pediatric Obesity

There has been a resurgence of interest in the contribution of genotype toward predisposition to obesity in humans. A variety of animal models (e.g., the ob/ob mouse and the fa/fa Zucker rat) have been described in which obesity is inherited as a simple Mendelian recessive trait. Such animals, which are members of highly inbred strains, demonstrate enhancements of metabolic efficiency within the first few weeks of life before the appearance of the relative hyperphagia that is part of their behavioral phenotype.^{417, 418} In no instance is the specific biochemical "lesion" that underlies these genetic obesities known. Though the elucidation of the molecular physiology of these fascinating examples of obesity will undoubtedly shed light on questions regarding the pathogenesis of human obesity, such insight will provide only a portion of the answer. In humans, obesity is clearly the result of complex interactions of genotype with potent environmental forces.

The apparent increase with age of the correlation between pediatric and adult adiposity may reflect progressively greater phenotypic expression of genetically programmed patterns of energy homeostasis with age. On the other hand, numerous postnatal environmental factors—including socioeconomic status, education, geographic location, family size, and of course diet—have also been correlated with subsequent risk of obesity.^{1, 419} In

this section, we discuss the relative contributions of various genetic and environmental factors to the development of pediatric obesity and its persistence into adulthood.

Genetics

Efforts to quantify the contribution of genotype to body habitus in humans have been of two general types. Comparisons of monozygotic and dizygotic twins are used to assess relative expression of identical and non-identical genotypes in presumably similar environments. Adoption studies, by comparing adoptees with their biologic and adoptive families, are another way to assess the relative contribution of genotype and environment to body composition. (Mueller⁴²⁰ provides a recent extensive review of the literature on the genetics of adiposity.)

Twin Studies

Studies of dizygous versus monozygous twins have suggested that as much as 80% of the variance in skinfold thickness or weight-for-height may be attributable to genotype.^{421, 422} Brook et al.⁴²³ compared the concordance of skinfold thicknesses in monozygous versus dizygous twins to assess the relative influence of genotype on phenotype in presumably similar environments. He reported that pooled heritabilities of triceps and subscapular skinfold thickness (the percent of variability in skinfold thicknesses that is attributable to genotype) rose from 50% in children less than 10 years of age to 98% in children over age 10. Bouchard⁴²⁴ reported that the concordance of fat mass and fat distribution was much higher between monozygous than dizygous twins. In the same study, intrapair concordances of lipoprotein lipase activity, maximal insulin-stimulated lipogenesis, and basal and stimulated rates of lipolysis were significantly higher within monozygous than dizygous twin pairs. Poehlman et al.⁴²⁵ studied AT metabolism in six pairs of adult nonobese monozygotic twins during a 22-day period of controlled overfeeding. While no consistent effects of overfeeding on *in vitro* basal or stimulated lipolysis or lipogenesis were noted, there was significant intrapair concordance for these measures. This implies that there may be genotypic influences on AT adaptation to caloric excess. Long-term studies have demonstrated that the heritability of percentage overweight remains relatively stable in monozygous twins examined at 20-year intervals. Since environments are presumably different for the twins, this suggests a strong genetic contribution to body habitus that is not an artifact of covariant environmental circumstances.^{422, 426} Yet, these data may also reflect persistence of feeding behaviors learned in common childhood surroundings.

Twin studies have been criticized for assuming that there is a constancy to the environment within dizygous twin pairs. Some twin studies also fail to take cognizance of possible important interactions of genotype and environment; there may be genotypic influences on how feeding behaviors,

etc., are influenced by the same environment. For example, studies of infant feeding behavior demonstrate that there are genetic influences on sucking patterns and sensitivity to sweetened formulas.^{127, 427, 428} The type of formula offered to the infant could thus have an impact on the phenotypic expression of this food preference genotype.

Adoption and Other Studies

Studies of adoptees as adults⁴²⁹ and as children⁴³⁰ have found significant correlations of BMI between female adoptees and their biologic but not adoptive mothers. Studies of male adoptees did not reveal significant differences in correlations of BMI with biologic versus adoptive fathers or mothers. It is not clear, however, to what degree the observed concordances between adoptees and their biologic but not adoptive parents reflect the heritability of adiposity versus LBM, i.e., whether fatness or leanness is inherited.⁴³¹ It should also be noted that some investigators have found equal correlations of skinfold thicknesses between adoptees and their biologic and adoptive parents.^{432, 433}

A number of investigators have attempted to locate genetic markers to identify those most likely to become obese. In a study of ten families with a strong family history of obesity, Apfelbaum et al.^{434, 435} found that the HLA B-18 antigen was present in 37% of obese individuals within the same family versus 10% of unrelated obese individuals and 14% of non-obese family members. The authors suggest that the HLA B-18 haplotype may be a marker of those at risk of obesity in certain types of familial obesity. These findings with regard to HLA haplotype have not (to our knowledge) been replicated. There are a variety of sampling artifacts that might explain such concordance of haplotype and corpulence. It is, however, possible that an "HLA-linked" gene controlling some aspect of energy homeostasis is involved. There is one report of virus-induced obesity in mice,⁴³⁶ probably on the basis of a subtle encephalopathy. Specific HLA-types predispose to certain infections, and it is at least conceivable that some instances of human obesity might be due to subclinical episodes of viral encephalopathy.

Environment

In 1968, Knittle and Hirsch⁴³⁷ reported that underfeeding or overfeeding of preweanling rat pups was associated with respective decreases or increases in body size and epididymal fat pad cellularity and cell size. These differences in body size and adiposity persisted despite subsequent ad libitum feeding of rat chow. Later demonstration that extreme obesity in humans is due to adipose hyperplasia as well as hypertrophy and of the irreducible nature of adipocyte number by weight loss¹⁷⁶ suggested that early nutritional factors might exert a strong influence on subsequent adiposity. Others have proposed that the human fetus is

relatively immune to the effects of maternal nutritional variation due to its ability to parasitize nutrients at the expense of the mother. While both of these hypotheses have had substantial effects on prenatal and postnatal feeding practices in humans, neither has been conclusively demonstrated.

As noted earlier, a metabolic tendency toward fat accretion in times of plenty as provision against times of famine may have been advantageous to our progenitors. It is unlikely that this tendency has changed substantially over the last generation, yet the prevalence of obesity continues to increase.⁴³⁸ This suggests that the rapidly changing environment has become increasingly permissive toward the phenotypic expression of this underlying tendency to accumulate adipose tissue.⁴³⁹ This section reviews various prenatal and postnatal factors that have been proposed as influences on childhood adiposity.

Prenatal Environmental Factors

Infant birthweight varies directly with maternal obesity and weight gain during pregnancy.^{180, 440–442} Rosso⁴⁴³ hypothesized that in the malnourished gravida, there is a restriction of the normal physiologic increases in maternal blood volume and/or cardiac output. This results in decreased placental size and blood flow, reduced nutrient transfer, and ultimately fetal growth retardation.

Prenatal overnutrition is best exemplified by the infant of the gestationally diabetic mother (IDM). Gestational diabetes mellitus exposes the fetus to high circulating glucose levels resulting in fetal hyperinsulinemia and increased lipogenesis. The relative obesity of the IDM is well known and has been inversely correlated with the degree of gestational glycemic control.^{444–446} Extrapolating from the work of Knittle and Hirsch cited earlier,⁴³⁷ an increased incidence of subsequent hypercellular obesity in the IDM might be anticipated.

There is, however, no consensus regarding the effects of gestational diabetes mellitus on long-term adiposity. Ginsberg-Fellner and Knittle⁴⁴⁷ found comparative hypercellularity of AT in IDM as infants, and several investigators have reported an increase in subsequent obesity.^{448–450} Evaluation of these phenomena is complicated by the fact that both the diabetic mother and her offspring may be manifesting a genetic predisposition toward obesity rather than an expression of prenatal environmental influences.

Studies of obesity-prone populations, such as the Pima Indians of Arizona, have endeavored to separate pregestational and gestational influences on subsequent adiposity. The Pimans represent a population known to be at high risk for obesity and maturity-onset diabetes mellitus. Pettit et al.⁴⁵¹ reported that a significantly higher percentage of the offspring of gestationally diabetic Pimans were obese at age 15 to 19 years than offspring of prediabetics or nondiabetics. These differences in the prevalence of subsequent obesity in offspring were independent of the degree of maternal

obesity. The positive correlation between maternal diabetes and adolescent obesity suggests that prenatal exposure of the fetus to the milieu of the diabetic mother may constitute an independent risk factor for subsequent obesity in this unique and relatively homogeneous (genetically) population.

Prenatal undernutrition in humans has, for obvious ethical reasons, been examined primarily through retrospective epidemiologic studies of populations subjected to prolonged periods of starvation as a result of man-made or natural disasters. Ravelli et al.⁴⁵² conducted a retrospective study of 307,700 19-year-old male military recruits conceived during the Nazi-imposed Dutch famine of 1944 to 1945. The prevalence of obesity (defined as weight/height greater than 120% of the World Health Organization [WHO] standards) was significantly higher among inductees whose mothers had been undernourished (estimated at 1,000 to 1,200 kcal/day) during the first two trimesters of pregnancy (2.77% versus 1.45% in controls from non-nutritionally deprived regions). Offspring of women undernourished during the third trimester of pregnancy, and who were subsequently underfed during the first few months of infancy, showed a significant reduction in birthweight and in the prevalence of subsequent obesity at age 19 (0.82% versus 1.32% in controls from non-nutritionally deprived regions of the country). Malnutrition in only the last trimester of pregnancy or the first year of life had no influence on the incidence of subsequent obesity. The authors suggest that the increased incidence of obesity in individuals subjected to early intrapartum nutritional deprivation might be due to an effect on developing hypothalamic centers regulating feeding and growth. The decreased incidence of obesity in those subjected to later undernutrition might reflect effects on intrauterine and extrauterine AT development. However, the statistical significance of the between-group differences reflects small differences in a large study population, and it is not clear whether these data reflect changes in LBM or actual AT mass.

Postnatal Environmental Factors

Studies of feeding beyond the neonatal period have only reemphasized the view that each child has a highly individual pattern of energy homeostasis and that there are no universally applicable dietary guidelines for the prevention of obesity. Formula-fed infants have been reported to be significantly longer and heavier than those who were breast-fed.⁴⁵³ However, no significant differences in BMI between members of these two groups were detectable at 8 years of age.⁴⁵⁴ Similarly, several other investigators have reported that subsequent adiposity is not significantly influenced by the age at which specific foods are introduced into the diet or other infant feeding practices.^{421, 455–457} Enzi et al.⁴⁵⁸ studied the growth of infants born to normal, obese, and diabetic mothers while attempting to keep all infants on 105 to 120 kcal/kg/day intake. Despite similar energy intakes, there were large variations in rates of AT deposition that were not predict-

able from pregravid maternal caloric intake or weight or a history of maternal diabetes. Butte et al.⁴⁵⁹ examined caloric intake and growth in 45 exclusively breast-fed infants and found that these children achieved adequate growth during the first 4 months of life despite caloric intakes that were significantly less than WHO-recommended daily allowances and approximately 25% less than the intake of formula-fed infants. The authors concluded that the infant may be capable of adjusting energy homeostatic systems to compensate for variations in the level of caloric intake. These studies are consistent with the concept of variable metabolic efficiency between individuals and stress that caloric requirements must be assessed on the basis of individual growth patterns.

Numerous other environmental variables have been implicated as factors that encourage expression of the obese genotype. In the United States, the prevalence of obesity is higher in children raised in urban than in rural communities and also varies between different geographic regions.⁴⁶⁰ (The National Health Examination Survey reported that obesity is most prevalent in the northeastern United States, followed by the Midwest, South, and West.⁴¹⁹) Children of larger families are less likely to be obese while children of single or of older parents are more likely to be obese.^{432, 461} Socioeconomic status and level of maternal education have been reported to vary inversely with preadolescent adiposity.^{432, 462} Dietz and Gortmaker⁴⁶³ reported significant association between the prevalence of obesity and the amount of time devoted to watching television in a large population study of children aged 6 to 17 years. They concluded that television viewing encourages inactivity and the consumption of calorically dense foods that then lead to the development of obesity. However, it is also possible that the obese child watches more television because of social stigmatization by peers or other consequences of obesity.

In summary, the phenotypic expression of genotypic influences on body composition appears to increase with age. Genotypic influences on adiposity can be expressed through feeding behavior and/or metabolic efficiency, but the specific differences in these systems between lean and obese individuals have not been clearly defined. Clearly, expression of genotype is influenced and can be overridden by environmental factors such as nutrient availability and need for physical activity. Ultimately, body composition, anatomic distribution of fat, and the systemic response to adiposity reflect a complex interaction of nature and nurture.

Prevention and Treatment

Studies by Zack et al.³¹⁹ suggest that childhood fatness may be more predictive of adolescent fatness than previously recognized. As previously discussed, there may be metabolic and behavioral characteristics of the child destined to become obese that are evident before the physical manifestations of obesity. If this is true, then earlier identification of the child at risk

of becoming an obese adult may be possible. However, there is little evidence that once such a child has been identified, a safe and long-term intervention can be made.^{464, 465} This section reviews current modes of therapy while emphasizing that, in addition to the tremendous difficulty in maintaining a weight-reduced state, there is a significant risk of therapy-related morbidity in the obese, growing child.

Early Therapeutic Intervention

As previously discussed, there is no evidence that early infant feeding practices influence the subsequent development of obesity. Studies of older children have addressed the issue of early therapeutic intervention in children who have already been identified as obese. In a study of obese pre-pubertal girls (mean age, 8.4 years), Hager et al.⁴⁶⁶ examined AT cellularity during a 1.5 to 1.9 year therapeutic trial of a 1,200 kcal/day diet and exercise regimen. They found that whereas the overall rate of fat accretion was diminished in these subjects, the increase in adipocyte number over the period of study was still greater than in age-matched lean controls. These data imply that hyperplasia of adipose tissue may be therapeutically restrained, but that the influence of genotypic or previous environmental factors that predisposed subjects toward a supranormal rate of appearance of adipocytes may persist despite clinically effective weight control.

Long-term studies of weight-reduced children have demonstrated rates of recidivism to obesity similar to those reported in adults. Lloyd et al.⁴⁶⁷ reported that 80% of a group of weight-reduced obese children had returned to their initial weight percentiles 9 years after weight reduction. Ginsberg-Fellner and Knittle⁴⁴⁷ examined 26 children who had been treated for obesity at age 2 to 10 years with dietary intervention leading to a 33% reduction in body weight expressed as percentage of "ideal." Seven to eight years later, all these children had returned to previous levels of obesity. Leibel and Hirsch⁴⁶ documented that many reduced-obese adults have a sharp (approximately 20%) and persistent decline in weight-maintenance energy requirements (corrected for body size). Despite that fact that they are weight stable, such individuals display a variety of symptoms (hunger, lethargy, depression, amenorrhea) and metabolic characteristics suggestive of a semi-fasted state. These changes in energy homeostasis, which accompany weight reduction, may reflect antecedent metabolic/behavioral characteristics and/or derangements induced by the weight loss process itself. In any event, these characteristics of the weight-reduced state clearly contribute to the high rate of recidivism among reduced-obese adults. The status of such systems in weight-reduced children has not been carefully examined.

Recommendations for Management

There are undoubtedly many different causes of obesity in children, and we currently lack sufficient knowledge to effectively prevent or treat most

of them. Obesity may be the manifestation of an underlying illness such as primary hyperinsulinism, excess cortisol secretion, hypothyroidism, Prader-Willi syndrome, or other syndromes of hyperphagia secondary to hypothalamic damage. However, these entities are rare in children. In the vast majority of cases, obesity is due to characteristics of eating behavior and/or energy expenditure that favor an increased body mass.

There are clear medical and psychosocial benefits to weight reduction for the obese. However, the Food and Drug Administration of the United States continues to report significant mortality-associated with low energy diets in adults and children.⁴⁶⁸⁻⁴⁷¹ Nylander⁴⁷² reported that 10% of teenagers in supervised weight control programs complained of at least three of the following symptoms: anxiety, depression, chilliness, constipation, amenorrhea, or mental sluggishness. Mallick^{473, 474} found that 80% of teenagers using unsupervised diets taken from popular books or magazines suffered from hunger, weakness, headaches, fatigue, nausea, constipation, nervousness, dizziness, poor concentration, altered menstrual function, and/or fainting. Numerous investigators^{475, 476} have reported secondary amenorrhea or dysmenorrhea with weight loss in adolescents. Adverse reactions to therapeutic dieting are as highly individualized as therapeutic responses, and subjects must be closely monitored during caloric restriction. While the psychosocial consequences of childhood obesity may seem paramount to the patients and their families, one must also consider the rigors of therapy and stigmatization of the child that may occur as a result of having their weight become the center of family attention.

The more radical treatments sometimes used for severely obese adults (jaw wiring, gastric stapling, jejunoileal bypass) are not recommended for children. The consequences of even moderate caloric restriction may include an impairment of statural or brain growth.^{262, 477} Thus, if possible, diet therapy should be directed toward slowing rates of fat accretion until body composition is normalized rather than toward restricting energy to the point of weight loss. All diets should be designed to provide sufficient quantities of carbohydrate, fat (especially essential fatty acids), protein, minerals, and vitamins to fully meet lean tissue growth requirements. "Special" diets consisting of drastically altered relative contents of carbohydrate, protein, or fat may be dangerous and yield no better results than a calorie-limited well-balanced diet.^{478, 479} Studies of highly restrictive protein-sparing diets in adolescents have suggested that impairment of protein metabolism with continued nitrogen losses may occur despite carbohydrate supplementation.⁴⁸⁰⁻⁴⁸²

The other critical component of therapy is exercise (Table 4). Contrary to popular conceptions, the obese child is not chronically physically underactive compared with lean peers.^{155, 483, 484} However, encouragement of exercise will allow the child to ingest more calories during the treatment period.

Studies of compliance of obese children with dietary regimens have emphasized the importance of a family-oriented approach to therapy. Refusal

TABLE 4.
Approximate Hourly Energy Expenditure of Adult
Men and Women During Exercise*

Level of Activity	Energy Expenditure (kcal/hr)	
	Men	Women
Light (walking at 2 miles per hour)	120–290	90–220
Moderate (running at 4 miles per hour)	300–450	250–350
Heavy (running at 5 miles per hour)	450–600	320–500

*Data are based on an average 65-kg man and 55-kg women. When corrected for lean body mass, values are not significantly different between sexes. Hourly energy expenditure depends on the individual activity as well as the age, body composition, and overall fitness of the individual.

to adhere to a weight-reduction plan may be the manifestation of other psychologic stresses. For example, Dietz and Gortmaker⁴⁶⁰ reported that children of married parents lose weight at higher rates than those of divorced parents. Once a weight-reduction plan has been recommended, conflicts frequently arise between the patient and nondieting family members regarding the degree of dietary restriction, who can eat different foods, etc.⁴¹⁹ If possible, the obese child and the entire family should adhere to a diet similar in composition if not quantity. Involvement of the entire family should help to minimize the feelings of isolation in the obese child.

In deciding upon a therapeutic approach, children should be classified with respect to apparent morbidity. Children who are obese (subscapular skinfold thickness > 75th percentile) should be evaluated for hypertension, hypercholesterolemia, and glucose intolerance. If none of these are present, then a weight-maintenance diet and exercise plan should be advised. The goal of such therapy is to allow the child to grow normally without a further increase in AT mass so that he or she will “outgrow” the obesity. While obesity at any time in life may predispose to adiposity-related morbidity (regardless of whether the obesity persists), the risks of weight reduction probably exceed the benefits in the otherwise healthy obese child. When adiposity-related morbidity is evident, more aggressive intervention is warranted. The hypertensive or diabetic child should attempt to reduce weight or alter body composition to the point that the morbidity is no longer evident within a 1-year period. The initial therapeutic approach should involve closely supervised diet and exercise plans. Some studies

have reported greater patient adherence to such treatments if they are administered in conjunction with some sort of behavior modification program.⁴⁸⁵ Because of the risks associated with attempts at weight reduction in children, such a plan should include the supervision of a dietician, as well as monitoring of school performance and frequent physical examination by the pediatrician. If the patient is unable to lose weight, and morbidity persists, then placement of the child in a highly supervised environment such as a weight-reduction summer camp should be considered. There is no documented role at present for pharmacotherapy in pediatric obesity, and surgical intervention should only be considered as a last resort in the morbidly obese child.

Obesity, or the propensity to become obese, is a highly heterogeneous disorder that is remarkably resistant to therapeutic intervention. The irreversibility of adipocyte hyperplasia and the increased likelihood of persistence of pediatric obesity into adulthood in the older child suggest that early detection of this propensity is highly desirable. The pediatrician can play a vital role in preventive medical care by closely monitoring patients and beginning simple dietary therapy before severe obesity develops. Such intervention is especially desirable in the case of children with a family history of adiposity-related morbidity. Once preventive or therapeutic intervention is begun, the child must be frequently evaluated to ascertain that normal linear and central nervous system growth continues. The adolescent, in particular, must be scrutinized for the possibility of precipitation of anorexia nervosa or bulimia.⁴⁸⁶

References

1. Garn SM: Continuities and changes in fatness from infancy through adulthood. *Curr Prob Pediatr* 1985; 15:1-47.
2. Johnson ML, Burke BS, Mayer J: The prevalence and incidence of obesity in a cross-section of elementary and secondary school children. *Am J Clin Nutr* 1956; 4:231-238.
3. Rauh JC, Schumsky DA, Witt MT: Height, weight, and obesity in urban school children. *Child Dev* 1967, pp 515-530.
4. Forbes GB, Amirhakimi GH: Skinfold thickness and body fat in children. *Hum Biol* 1970; 42:401-418.
5. Brownell KD: New developments in the treatment of obese children and adolescents, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984, pp 175-184.
6. Kleinfeld NR: The ever fatter business of thinness. *The New York Times*, Sept 7, 1986, p C:1.
7. Personal Communication, 1987.
8. Neel JV: Diabetes mellitus: A "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genetics*, 1962; 14:353-362.
9. Frisch RE, McArthur JW: Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science* 1974; 185:949-951.

10. Siri W: Body composition from fluid spaces and density: Analysis of methods, in Brozek J, Herschel A (eds): *Techniques for Measuring Body Composition*. Washington, DC, National Academy of Science, 1961, pp 223–244.
11. Segal KR, Gutin B, Presta E, et al: Estimation of body composition by electrical impedance methods: A comparative study. *J Appl Physiol* 1985; 58:1565–1571.
12. Brozek J, Grande F, Anderson JT, et al: Densitometric analysis of body composition: Revision of some quantitative assumptions. *Ann NY Acad Sci* 1963; 110:113–140.
13. Behnke AR, Wilmore JH: *Evaluation of Body Build and Composition*. Englewood Cliffs, NJ, Prentice-Hall, 1974.
14. Jackson AS, Pollock ML: Generalized equations for predicting body density of men. *Br J Nutr* 1978; 40:497–504.
15. Ku LC, Shapiro LR, Crayford PB, et al: Body composition and physical activity in 8-year-old children. *Am J Clin Nutr* 1981; 34:2770–2775.
16. Dauncey MJ, Gillian G, Gairdner D: Skinfold thickness measurements. *Arch Dis Child* 1977; 52:223–227.
17. Fomon SJ, Haschke F, Ziegler EE, et al: Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* 1982; 35:1169–1175.
18. Friis-Hansen B: Body water compartments in children: Changes during growth and related changes in body composition. *Pediatrics* 1961; 28:169–181.
19. Parizkova J, Buzkova P: Relationship between skinfold thickness measured by harpenden caliper and densitometric analysis of total body fat in men. *Human Biol* 1971; 43:16–21.
20. Womersley J, Boddy K, King PC, et al: A comparison of the fat-free mass of young adults estimated by anthropometry, body density, and total body potassium. *Clin Sci* 1972; 43:469.
21. Pierson RN, Wang JC, Thornton JC, et al: Body potassium by four-pi 40K counting: An anthropometric correction. *Am J Physiol* 1984; 246:F234.
22. Presta E, Wang J, Harrison GG, et al: Measurement of total body electrical conductivity: A new method for estimation of body composition. *Am J Clin Nutr* 1983; 37:735–739.
23. Presta E, Segal KR, Gutin B, et al: Comparison in man of total body electrical conductivity and lean body mass derived from body density: Validation of a new body composition method. *Metabolism* 1983; 32:524–527.
24. Presta E, Costa R, Slonim A, et al: Body composition in adolescents: Estimation by total body electrical conductivity. *Am J Physiol*, 1988; in press.
25. Florey CV: The use and interpretation of ponderal index and other weight-height ratios in epidemiologic studies. *J Chron Dis* 1970; 23:93–103.
26. Roche AF, Sievogel RM, Chumlea WC: Grading body fatness from limited anthropometric data. *Am J Clin Nutr* 1981; 34:2831–2838.
27. Harrison GG: Height-weight tables. *Ann Int Med* 1985; 103:989–994.
28. Harlan WR, Hull AL, Schmouder RP, et al: Dietary intake and cardiovascular risk factors: Part I. Blood pressure correlates. *Vital Health Stat* 1983; series 11, no 226.
29. Heald FP, Hunt EE, Schwartz R, et al: Measures of body fat and hydration in adolescent boys. *Pediatrics* 1963; 31:226.
30. Young CM, Bogan AD, Roe DA, et al: Body composition of preadolescent and adolescent girls : IV. Body water and creatinine. *J Am Diet Assoc* 1968; 53:579.

31. Seltzer CC, Goldman RF, Mayer J: The triceps skinfold as a predictive measure of body density and body fat in obese adolescent girls. *Pediatrics* 1965; 36:212.
32. Dumin JVGA, Rahaman NM: Assessment of the amount of fat in the human body from measurement of skinfold thickness. *Br J Nutr* 1967; 21:681.
33. Johnson CL, Fulwood R, Abraham S, et al: Basic data on anthropometric measurements and angular measurements of the hip and knee joints for selected age groups 1–74 years of age. *Vital Health Stat* 1981; 11(219):1–9.
34. Cronk CE, Roche AF: Race- and sex-specific reference data for triceps and subscapular skinfolds and weight/stature. *Am J Clin Nutr* 1982; 35:347–354.
35. Williams DRR: Relationship between skinfold thickness and serum triglyceride concentrations. *Guy's Hosp Rep* 1973; 122:331–333.
36. Despres JP, Allard C, Tremblay A, et al: Evidence for a regional component of body fatness in the association with serum lipids in men and women. *Metabolism* 1985; 34:967–973.
37. Dahlstrom S, Viikari J, Akerblom HK, et al: Atherosclerosis precursors in Finnish children and adolescents: II. Height, weight, body mass index, and skinfolds, and their correlation to metabolic variables. *Acta Paediatr Scand* 1985; 318:65–78.
38. Albrink MJ, Meigs JW: Interrelationship between skinfold thickness, serum lipids, and blood sugar in normal men. *Am J Clin Nutr* 1964; 15:255–261.
39. Feldman R, Sender AJ, Siegelaub AB: Differences in diabetic and nondiabetic fat distribution patterns by skinfold measurements. *Diabetes* 1969; 18:478–486.
40. Micozzi MS, Albanes D, Jones DY, et al: Correlations of body mass indices with weight, stature, and body composition in men and women in NHANES I and II. *Am J Clin Nutr* 1986; 45(1):7–13.
41. Roche AF: Recent advances in child growth and development. *Prog Clin Biol Res* 1981; 59A:321–329.
42. Abdel-Malek AK, Mukherjee D, Roche AF: A method of constructing an index of obesity. *Hum Biol* 1985; 57:415–430.
43. Apfelbaum M, Bostaron J, Lacatis D: Effect of caloric restriction and excessive caloric intake on energy expenditure. *Am J Clin Nutr* 1971; 24:1405–1409.
44. Sims EAH, Danforth E, Horton ES, et al: Endocrine and metabolic effects of experimental obesity in man. *Rec Prog Horm Res* 1973; 29:457–496.
45. Sims EAH, Danforth E: Expenditure and storage of energy in man. *J Clin Invest* 1987; 79:1019–1025.
46. Leibel RL, Hirsch J: Diminished energy requirements in reduced-obese patients. *Metabolism* 1984; 33:164–170.
47. Bray GA, and York DA: Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis. *Physiol Rev* 1979; 59:719–809.
48. Brobeck JR, Tepperman J, Long CNH: Experimental hypothalamic hyperphagia in the albino rat. *Yale J Biol Med* 1943; 15:831–833.
49. Bernardis LL, Frohman LA: Effects of hypothalamic lesions at different loci on development of hyperinsulinemia and obesity in the weanling rat. *J Comp Neur* 1971; 141:107–116.
50. Stellar E: Neural basis: Introduction, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984, pp 1–4.

51. Leibowitz SF, Rossakis C: Pharmacological characterization of perifornical hypothalamic beta-adrenergic receptors mediating feeding inhibition in the rat. *Neuropharmacology* 1978; 17:691-701.
52. Leibowitz SF: Reciprocal hunger-relating circuits involving alpha- and beta-adrenergic receptors located, respectively, in the ventromedial and lateral hypothalamus. *Proc Natl Acad Sci USA* 1970; 67:1063-1070.
53. Margules DL: Alpha-adrenergic receptors in the hypothalamus for the suppression of feeding behavior by satiety. *J Comp Physiol Psychol* 1970; 73:1-12.
54. Leibowitz SF: Neurochemical systems of the hypothalamus: Control of feeding and drinking behavior and water-electrolyte excretion, in Morgane PJ, Panksepp J (eds): *Handbook of the Hypothalamus*. Vol 3a: *Behavioral Studies of the Hypothalamus*. New York, Marcel Dekker, 1980, pp 299-437.
55. Siggins GR: Catecholamines and endorphins as neurotransmitters and neuromodulators, in Tapia R, Cotman CW(eds): *Regulatory Mechanisms of Synaptic Transmission*. New York, Plenum Press, 1981, pp 1-27.
56. Hoebel BG: Neurotransmitters in the control of feeding and its rewards: Monoamines, opiates, and brain-gut peptides, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984, pp 15-38.
57. Sclafani A: The role of hyperinsulinemia and the vagus nerve in hypothalamic hyperphagia reexamined. *Diabetologia* 1981; 20:402-410.
58. Sclafani A, Springer D: Dietary obesity in adult rats: Similarities to hypothalamic and human obesity syndromes. *Physiol Behav* 1976; 17:461-471.
59. Franklin KBJ, Herberg LJ: Ventromedial syndrome: The rats "finickiness" results from the obesity, not from the lesions. *J Comp Physiol Psychol* 1974; 87:410-414.
60. Watson PJ, Cox VS: An analysis of barbiturate-induced eating and drinking in the rat. *Physiol Psychol* 1976; 4:325-332.
61. Robinson RG, McHugh PR, Bloom FE: Chlorpromazine-induced hyperphagia in the rat. *Psychopharmacol Comm* 1975; 1:37-50.
62. Bray GA, York DA: Studies on food intake in genetically obese rats. *Am J Physiol* 1972; 223:176-179.
63. Antelman SM, Black CA, Rowland NE: Clozapine induces hyperphagia in undrugged rats. *Life Sci* 1977; 2:1747-1750.
64. Booth DA, Jarmen SP: Ontogeny and insulin-dependence which follows carbohydrate absorption in the rat. *Behav Biol* 1975; 15:159-172.
65. Baxter MG, Miller AA, Soroko FE: The effect of cyproheptidine of food consumption in the fasted rat. *Br J Pharmacol* 1970; 39:229-230.
66. Blundell JE, Leshem MB: The effect of serotonin manipulation in rats with lateral hypothalamic lesions, in *Proceedings, Fifth International Congress on Physiology of Food and Fluid Intake*, Jerusalem, 1974.
67. Sanger DJ, McCarthy PS: Differential effects of morphine on food and water intake in food deprived and free-feeding rats. *Psychopharm* 1980; 72:103-106.
68. Panksepp J, Pollack A, Krost K, et al: Feeding in response to repeated prothamine zinc insulin injections. *Physiol Behav* 1975; 14:493-497.
69. Rowland DL, Perrings TS, Thommes JA: Comparison of androgenic effects on food intake and body weight in adult rats. *Physiol Behav* 1980; 24:205-209.
70. Lorenz D, Nardi P, Smith GP: Atropine methyl nitrate inhibits sham feeding in the rat. *Pharmacol Biochem Behav* 1978; 8:405-407.

71. Dobrzanski S, Doggett NS: The effects of (+)-amphetamine and fenfluramine on feeding in starved and satiated mice. *Psychopharm* 1976; 48:283–286.
72. Blundell JE, Latham CJ: Serotonergic influences of food intake: Effect of 5-hydroxytryptophan on parameters of feeding behavior in deprived and free-feeding rats. *Pharmacol Biochem Behav* 1979; 11:431–437.
73. Wade G: Some effects of ovarian hormones on food intake and body weight in female rats. *J Comp Physiol Psychol* 1975; 85:193.
74. Gibbs J, Young RC, Smith GP: Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* 1973; 245:323–335.
75. Holtzman SG: Behavioural effects of separate and combined administration of naloxone and d-amphetamine. *J Pharmacol Exp* 1974; 189:51–60.
76. Oomura Y, Shimizu N, Miyahara S, et al: Chemosensitive neurons in the hypothalamus: Do they relate to behavior? in Hoebel BG, Novin D (eds): *The Neural Basis of Feeding and Reward*. Brunswick, Maine, Haer Institute, 1982, pp 551–566.
77. Russek M, Mogenson GJ, Stevenson JAF: Calorigenic, hyperglycemic, and anorexigenic effects of adrenaline and noradrenaline. *Physiol Behav* 1967; 2:429–433.
78. Smith GP: Gut hormone hypothesis of postprandial satiety, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984, pp 67–76.
79. Wurtman RJ, Wurtman JJ: Nutrients, neurotransmitter synthesis, and the control of food intake, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984, pp 77–87.
80. Myers RD, McCaleb ML: Feeding: Satiety signal from intestines triggers brain's noradrenergic mechanism. *Science* 1980; 209:1035–1037.
81. McCaleb ML, Myers RD: Cholecystokinin acts on the hypothalamic "norepinephrine system" involved in feeding. *Peptides* 1980; 1:47–49.
82. Rolls ET: Feeding and reward, in Hoebel BG, Novin D (eds): *The Neural Basis of Feeding and Reward*. Brunswick, Maine, Haer Institute, 1982, pp 323–338.
83. Nishino H, Ono T, Fuduka M, et al: Lateral hypothalamic neuron activation during monkey var press feeding behavior: Modulation by glucose, morphine, and naloxone, in Hoebel BG, Novin D (eds): *The Neural Basis of Feeding and Reward*. Brunswick, Maine, Haer Institute, 1982, pp 355–373.
84. Pittman QJ, Hatton JD, Bloom FE: Morphine and opioid peptides reduce paraventricular neuronal activity: Studies on the rat hypothalamic slice preparation. *Proc Natl Acad Sci USA* 1980; 77:5527–5531.
85. Muehlethaler M, Gaehwiler BH, Dreifuss JJ: Enkephalin-induced inhibition of hypothalamic paraventricular neurons. *Brain Res* 1980; 197:264–268.
86. Trygstad O, Foss I, Edminson PD: Chromatographic patterns of urinary peptides in anorexia nervosa. *Acta Endocrinol* 1978; 89:196–208.
87. Sofia RD, Barry H: Acute and chronic effects of Δ^9 -tetrahydrocannabinol on food intake by rats. *Psychopharmacologia* 1974; 39:213–222.
88. Davis JD, Gallagher TL, Ladove R: Food intake controlled by a blood factor. *Science* 1967; 156:1247–1248.
89. Glick Z: Food intake of rats administered with glycerol. *Physiol Behav* 1980; 25:621–626.
90. Samanin R, Caccia S, Bendotti C, et al: Further studies on the mechanism of serotonin-dependent anorexia in rats. *Psychopharm* 1980; 68:99–104.

91. Smith GP, Epstein AN: Increased feeding in response to decreased glucose utilization in the rat and monkey. *Am J Physiol* 1969; 217:1083–1087.
92. Likuski HJ, Debons AF, Cloutier RJ: Inhibition of gold thioglucose-induced hypothalamic obesity by glucose analogues. *Am J Physiol* 1967; 212:669–676.
93. Novin D: Visceral mechanisms in the control of food intake, in Novin D, Wyrwicka W, Bray GA (eds): *Hunger: Basic Mechanisms and Clinical Implications*. New York, Raven Press, 1976, pp 357–369.
94. Larue-Achagiotis C, LeMagnen J: Effect of long-term intravenous insulin infusion on body weight and food intake by intravenous versus intraperitoneal routes. *Appetite* 1985; 6:319–329.
95. VanderWeele D: The alimentary canal, liver, and vagus play a role in short-term feeding: But is the role regulation, correlation, glucostasis, or spurious association. *Int J Obes* 1985; 8:51–64.
96. Geary N, Langhous W, Scherrar E: Metabolic concomitants of glucagon-induced suppression of feeding in the rat. *Am J Physiol* 1981; 241:R330–335.
97. Stanley BG, Leibowitz SF: Regulation of feeding behavior by hypothalamic neuropeptide and polypeptide YY. *Proc Neurol Endocrinol Peptides Recep Symp*, Washington, DC, 1985, p 67.
98. Stanley BG, Leibowitz SF: Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior. *Proc Natl Acad Sci USA* 1985; 82:3940–3943.
99. Leibowitz SF: Hypothalamic catecholamine systems in relation to continuity of eating behavior and mechanisms of reward, in Hoebel BG, Novin D (eds): *The Neural Basis of Feeding and Reward*. Brunswick, Maine, Haer Institute 1982, pp 241–258.
100. Louis-Sylvestre J, LeMagnen J: Palatability and preabsorptive insulin release. *Neurosci Biobehav Rev* 1980; 4:43–46.
101. Doggett NS, Jawaharlal K: Some observations on the anorectic activity of prostaglandin F_{2α}. *Br J Pharmacol* 1977; 60:409–415.
102. Doggett NS, Jawaharlal K: Anorectic activity of prostaglandin precursors. *Br J Pharmacol* 1977; 60:417–423.
103. Atkinson J, Kirchertz E, Peters-Haefeli L: Effect of peripheral clonidine on ingestive behavior. *Physiol Behav* 1978; 21:73–77.
104. Lotter EC, Krinsky R, McKay JM, et al: Somatostatin decreases food intake of rats and baboons. *J Comp Physiol Psychol* 1981; 95:278–287.
105. Leibowitz SF: Ingestion in the satiated rat: Role of alpha and beta receptors in mediating effects of hypothalamic adrenergic stimulation. *Physiol Behav* 1975; 14:743–754.
106. Gray JM, Wade GN: Food intake, body weight, and adiposity in female rats: Actions and interactions of progestins and antiestrogens. *Am J Physiol* 1981; 240:E474–481.
107. King BM, Phelps GR, Frohman LA: Hypothalamic obesity in female rats in absence of vagally mediated hyperinsulinemia. *Am J Physiol* 1980; 239:E437–441.
108. Hales CN, Kennedy GC: Plasma glucose, non-esterified fatty acid and insulin concentrations in hypothalamic hyperphagic rats. *Biochem J* 1964; 90:620–624.
109. Louis-Sylvestre J: Preabsorptive insulin release and hypoglycemia in rats. *Am J Physiol* 1976; 230:E56–60.

110. Louis-Sylvestre J: Relationship between two states of prandial insulin release in rats. *Am J Physiol* 1978; 233:E103-111.
111. Steffens AB: The modulatory effect of the hypothalamus on glucagon and insulin secretion in the rat. *Diabetologia* 1981; 20:411-416.
112. Grossman SP, Grossman L: Ionophoretic injections of kainic acid into the rat lateral hypothalamus: Effects on ingestive behavior. *Physiol Behav* 1983; 29:553-559.
113. Carpenter RG, King BM, Stamoutsos BA, et al: VMH lesions in vagotomized rats: A note of caution. *Physiol Behav* 1978; 21:1031-1035.
114. Carpenter RG, Stamoutsos BA, Dalton LD, et al: VMH obesity reduced but not reversed by scopolamine methyl nitrate. *Physiol Behav* 1979; 23:955-959.
115. Keesey RE, Corbett SW: Metabolic defense of the body weight set-point, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984, pp 77-96.
116. Koh SD, Teitlebaum P: Absolute behavioral taste thresholds in the rat. *J Comp Physiol Psychol* 1961; 54:823-829.
117. Stevenson JAF, Montemurro DG: Loss of weight and metabolic rate of rats with lesions in the medial and lateral hypothalamus. *Nature* 1963; 198:92.
118. Ferguson NBL, Keesey RE: Effect of a quinine adulterated diet upon body weight maintenance in male rats with ventromedial hypothalamic lesions. *J Comp Physiol Psychol* 1975; 478-488.
119. Cox JE, Powley TL: Intragastric pair feeding fails to prevent VMH obesity or hyperinsulinemia. *Am J Physiol* 1981; 240:E566-572.
120. Grossman SP: Contemporary problems concerning our understanding of brain mechanisms that regulate food intake and body weight, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorders*. New York, Raven Press, 1984; pp 5-14.
121. Grossman SP: The VMH: A center for affective reaction, satiety, or both. *Physiol Behav* 1966; 1:1-10.
122. Stricker EM, Swerdloff AF, Zigmond MJ: Intrahypothalamic injections of kainic acid produce feeding and drinking deficits in rats. *Brain Res* 1976; 158:470-473.
123. Hernandez L, Hoebel BG: Overeating after midbrain 6-hydroxydopamine: Prevention by central injection of selective reuptake blockers. *Brain Res* 1982; 245:333-343.
124. Bray GA: Obesity-A disease of nutrient or energy balance? *Nutr Rev* 1987; 45:33-43.
125. Drewnowski A, Brunzell JD, Sande K, et al: Sweet tooth reconsidered: Taste responsiveness in human obesity. *Physiol Behav* 1985; 35:617-622.
126. Nisbett RE, Hanson LR, Harris A, et al: Taste responsiveness, weight loss, and the ponderostat. *Physiol Behav* 1974; 11:641-645.
127. Agras WS, Kraemer HC, Berkowitz RI, et al: Does a vigorous feeding style influence early development of adiposity? *J Pediatr* 1987; 110:799-804.
128. Drabman RS, Cordua G, Hammer D, et al: Developmental trends in eating rates of normal and overweight preschool children. *Child Dev* 1979; 50:211-220.
129. Adams N, Ferguson J, Stunkard AJ, et al: The eating behavior of obese and nonobese women. *Behav Res Ther* 1978; 16:225-236.
130. Garrow JS: Energy balance in man: An overview. *Am J Clin Nutr* 1987; 45:1114-1119.

131. Garrow JS: *Energy Balance and Obesity in Man*. Amsterdam, Holland Biomedical Press, 1978.
132. Jequier E: Energy expenditure in obesity. *Clin Endocrinol Metab* 1984; 13:563–580.
133. Newsholme EA, Crabtree B: Substrate cycles in metabolic regulation and in heat generation. *Biochem Soc Symp* 1976; 41:61–109.
134. Katz J, Rognstad R: Futile cycles in the metabolism of glucose. *Curr Top Cell Regul* 1976; 10:237–289.
135. Newsholme EA: Substrate cycles: Their metabolic, energetic, and thermic consequences in man. *Biochem Soc Symp* 1978; 43:183–205.
136. Wolfe RR, Herndon RN, Jahoor F, et al: Effect of severe burn injury on substrate cycling by glucose and fatty acids. *N Engl J Med* 1987; 317:403–408.
137. Newsholme EA: A possible metabolic basis for the control of body weight. *N Engl J Med* 1980; 302:400–405.
138. Cahill GF: Metabolic memory. *N Engl J Med* 1980; 302:396–397.
139. Keys A, Brozek J, Henschel A: *The Biology of Human Starvation*. Minneapolis, University of Minnesota Press, 1950.
140. Rothwell NJ, Stock MJ: A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 1979; 281:31–35.
141. Miller DS, Mumford P, Stock MJ: Gluttony: II. Thermogenesis in overeating man. *Am J Clin Nutr* 1967; 20:1223–1229.
142. Neumann RO: Experimentelle Beiträge zur Lehre von dem täglichen Nahrungsbedarf des Menschen unter besonderer Berücksichtigung der notwendigen Eiweißmenge. *Arch Hyg* 1902; 45:1–87.
143. Gulick A: A study of weight regulation in the adult human body during overnutrition. *Am J Physiol* 1922; 60:271–295.
144. Landsberg L, Young JB: The role of the sympathoadrenal system in modulating energy expenditure. *Clin Endocrinol Metab* 1984; 13:475–500.
145. Danforth E, Burger A: The role of thyroid hormones in the control of energy expenditure. *Clin Endocrinol Metab* 1984; 13:581–596.
146. Danforth E, Horton ES, O'Connell M, et al: Dietary-induced alterations in thyroid hormone metabolism during overnutrition. *J Clin Invest* 1979; 64:1336–1347.
147. Vagenakis AG, Burger A, Portnoy GI, et al: Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting. *J Clin Endocrinol Metab* 1975; 41:191–194.
148. Wimpfheimer C, Saville E, Voirol MJ, et al: Starvation-induced decreased sensitivity of resting metabolic rate to triiodothyronine. *Science* 1979; 205:1272–1273.
149. Welle SL, Campbell RG: Decrease in resting metabolic rate during rapid weight loss is reversed by low dose thyroid hormone treatment. *Metabolism* 1986; 35.
150. Young JB, Landsberg L: Suppression of the sympathetic nervous system during fasting. *Science* 1977; 196:1473–1475.
151. Young JB, Landsberg L: Stimulation of the sympathetic nervous system during sucrose feeding. *Nature* 1977; 269:615–617.
152. Ravussin E, Burnand B, Schutz Y, et al: Twenty-four hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. *Am J Clin Nutr* 1982; 35:566–573.

153. Halliday D, Hesp R, Stalley SF, et al: Resting metabolic rate, weight, surface area, and body composition in obese women. *Int J Obes* 1979; 3:1–6.
154. James WPT, Bailes J, Davies HL, et al: Elevated metabolic rates in obesity. *Lancet* 1978; i:1122–1125.
155. Stefanik PA, Heald FP, Mayer J: Caloric intake in relation to energy output of obese and nonobese adolescent boys. *Am J Clin Nutr* 1959; 7:55–61.
156. McCarthy MG: Dietary and activity patterns of obese women in Trinidad. *J Am Diet Assoc* 1966; 48:33–37.
157. Schutz Y, Ravussin E, Diethelm R, et al: Spontaneous physical activity measured by radar in obese and control subjects in a respiration chamber. *Int J Obes* 1982; 6:23–28.
158. Dore C, Hesp R, Wilkins D, et al: Prediction of energy requirements of obese patients after massive weight loss. *Hum Nutr Clin Nutr* 1982; 36C:41–48.
159. Golay A, Schutz Y, Meyer H: Glucose induced thermogenesis in non-diabetic and diabetic obese subjects. *Diabetes* 1982; 31:1023–1028.
160. Sorbis R, Monti M, Nilsson-Ehle P, et al: Heat production by adipocytes from obese subjects before and after weight reduction. *Metabolism* 1982; 31:973–978.
161. Schwartz RS, Halter JB, Bierman E: Reduced thermic effect of feeding in obesity: Role of norepinephrine. *Metabolism* 1983; 32:114–117.
162. Pittet PL, Chappuis PL, Acheson K, et al: Thermic effect of glucose in obese subjects studied by direct or indirect calorimetry. *Br J Nutr* 1976; 35:281–282.
163. Contaldo F, Presta E, di Biase G, et al: Preliminary evidence for brown fat defect in human obesity, in Cioffi LA (ed): *The Body Weight Regulatory System: Normal and Disturbed Mechanisms*. New York, Raven Press, 1981, pp 143–146.
164. Kaplan M, Leveille GA: Calorigenic response in obese and nonobese women. *Am J Clin Nutr* 29:1108–1113.
165. Jung RT, Shetty WP, James WPT: Reduced thermogenesis in obesity. *Nature* 1979; 279:322–333.
166. Bessard T, Schutz Y, Jequier E: Energy expenditure and postprandial thermogenesis in obese women before and after weight loss. *Am J Clin Nutr* 1982; 38:680–693.
167. Sharief NN, MacDonald I: Differences in dietary-induced thermogenesis with various carbohydrates in normal and overweight men. *Am J Clin Nutr* 1982; 35:267–272.
168. Welle SL, Campbell RG: Normal thermic effect of glucose in obese women. *Am J Clin Nutr* 1983; 37:87–92.
169. Katzeff HL, O'Connell M, Horton ES Jr, et al: Metabolic studies in human obesity during overnutrition and undernutrition: Thermogenic and hormonal responses to norepinephrine. *Metabolism* 1986; 35:166–175.
170. Felig P, Cunningham J, Levitt M, et al: Energy expenditure in obesity in fasting and postprandial state. *Am J Physiol* 1983; 244:E45–51.
171. Jequier E: Does a thermogenic defect play a role in the pathogenesis of human obesity. *Clin Physiol* 1983; 3:1–7.
172. Keesey RE, Boyle PC, Kemnitz JW, et al: The role of the lateral hypothalamus in determining the body weight set-point, in Novin D, Wyrwicka W, Bray GA (eds): *Hunger: Basic Mechanisms and Clinical Implications*. New York, Raven Press, 1975, pp 243–245.

173. Knittle JL: Adipose tissue development in man, in Faulkner F, Tanner JM (eds): *Human Growth*. Vol 2: *Postnatal Growth*. New York, Plenum Press, 1978, pp 295–315.
174. Hirsch J, Faust IM, Johnson PR: What's new in obesity: Current understanding of adipose tissue morphology, in Freinkel N (ed): *Contemporary Metabolism*, vol 1. New York, Plenum Press, New York, 1978, pp 385–399.
175. Bjorntorp P, Sjostrom L: Number and size of adipose tissue fat cells in relation to metabolism in human obesity. *Metabolism* 1971; 20:703–713.
176. Hirsch J, Batchelor B: Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab* 1976; 5:299–311.
177. Salans LB, Cushman SW, Weismann RE: Adipose cell size and number in nonobese and obese patients. *J Clin Invest* 1973; 52:929–941.
178. Bagdade JD, Hirsch J: Gestational and dietary influences on the lipid content of the infant buccal fat pad. *Proc Soc Exp Biol Med* 1966; 122:616–619.
179. Leibel RL, Berry EM, Hirsch J: Biochemistry and development of adipose tissue in man, in Conn HL Jr, DeFelice EA, Kuo P (eds): *Health and Obesity*. New York, Raven Press, 1983, pp 21–48.
180. Rosso P: Prenatal nutrition and fetal growth and development. *Pediatr Ann* 1981; 10:21–30.
181. Novak LP: Total body water and solids in six- to seven-year old children: Differences between the sexes. *Pediatrics* 1966; 38:483–490.
182. Owen GM, Jensen RL, Fomon SJ: Sex-related differences in total body water and exchangeable chloride during infancy. *J Pediatr* 1962; 60:858–866.
183. Flynn MA, Murthy Y, Clark J, et al: Body composition of negro and white children. *Arch Environ Health* 1970; 20:604–619.
184. Forbes GB: Body composition in adolescence, in Faulkner F, Tanner JM (eds): *Human Growth: A Comprehensive Treatise*, vol 2. New York, Plenum Press, 1986, pp 119–145.
185. Friis-Hansen B: Body composition during growth. *Pediatrics* 1971; 47:264–271.
186. Frisch RE, Revelle R, Cook S: Components of weight at menarche and the initiation of the adolescent growth spurt in girls: Estimated total body water, lean body weight, and fat. *Hum Biol* 1973; 45:469–483.
187. Holliday MA: Body composition and energy needs during growth, in Faulkner F, Tanner JM (eds): *Human Growth: A Comprehensive Treatise*, vol 2. New York, Plenum Press, 1986, pp 101–123.
188. Hager A, Sjostrom L, Arvidsson B, et al: Body fat and adipose tissue cellularity in infants: A longitudinal study. *Metabolism* 1977; 26:607–614.
189. Brook CGD: Cellular growth: Adipose tissue, in Faulkner F, Tanner JM (eds): *Human Growth*. Vol 2: *Post-natal Growth*. New York, Plenum Press, 1978, pp 21–33.
190. Knittle JL, Timmers K, Ginsberg-Fellner F, et al: The growth of adipose tissue in children and adolescents. *J Clin Invest* 1979; 63:239–246.
191. Klyde BJ, Hirsch J: Increased cellular proliferation in adipose tissue of adult rats fed a high-fat diet. *J Lipid Res* 1979; 20:705–715.
192. Faust IM, Miller WH Jr: *Hyperplastic Growth of Adipose Tissue in Obesity*. New York, Raven Press, 1983, pp 41–51.
193. Richardson RL, Hausman GJ, Campion DR: Response of pericytes to thermal lesion in the inguinal fat pad of 10 day old rats. *Acta Anat* 1982; 114:41–52.

194. Hausman GJ, Richardson RL: Cellular and vascular development in immature rat adipose tissue. *J Lipid Res* 1983; 24:522–532.
195. Hausman GJ, Campion DR, Martin RJ: Search for the adipocyte precursor cell and factor that promote its differentiation. *J Lipid Res* 1980; 21:657–669.
196. Larsen T, Myhre K, Vik-Mo H, et al: Adipose tissue perfusion and fatty acid release in exercising rats. *Acta Physiol Scand* 1981; 113:111–116.
197. Crandall DL, Goldstein BM, Huggins F, et al: Adipocyte blood flow: Influence of age, anatomic location, and dietary manipulation. *Am J Physiol* 1984; 247:R46–R51.
198. Nielsen SL, Bitsch V, Larsen OA, et al: Blood flow through human adipose tissue during lipolysis. *Scand J Clin Lab Invest* 1968; 22:124–130.
199. Martin SE, Bockman EL: Adenosine regulates blood flow and glucose uptake in adipose tissue of dogs. *Am J Physiol* 1986; 250:H1127–1135.
200. Prinz WA, West DB, Cohen A, et al: Intraarterial insulin infusion reduces adipose tissue blood flow (ATBF). *Fed Proc Abs* 1986; 45(3):2599.
201. Ailhaud G: Adipose cell differentiation in culture. *Mol Cell Biochem* 1982; 49:17–31.
202. Greenberger JS: Corticosteroid-dependent differentiation of human marrow preadipocytes in vitro. *In Vitro* 1979; 15:823–828.
203. Gharbi-Chihi J, Grimaldi P, Torresani J, et al: Triiodothyronine and adipose conversion of ob17 preadipocytes: Binding to high affinity sites and effects on fatty acid synthesizing and esterifying enzymes. *J Receptor Res* 1981; 2:153–173.
204. Negrel R, Grimaldi P, Ailhaud G: Differentiation of ob17 preadipocytes to adipocytes: Effects of prostaglandin $F_{2\alpha}$ and relationship to prostaglandin synthesis. *Biochem Biophys Acta* 1981; 666:15–24.
205. Min HY, Spiegelman BM: Adipsin, the adipocyte serine protease: Gene structure and control of expression by tumor necrosis factor. *Nucleic Acids Res* 1986; 14(22):8879–8892.
206. Hunt CR, Ro JH, Dobson DE, et al: Adipocyte P2 gene: Developmental expression and homology of 5'-flanking sequences among fat cell-specific genes. *Proc Nat'l Acad Sci USA* 1986; 83(11):3786–3790.
207. Faust IM, Johnson PR, Stern JS, et al: Diet induced adipocyte number increase in adult rats: A new model of obesity. *Am J Physiol* 1978; 235:E279–E286.
208. Bjorntorp P: Adipocyte precursor cells, in Bjorntorp P, Cairella M, Howard AN (eds): *Recent Advances in Obesity Research III*. London, John Libbey, 1981, pp 58–69.
209. Cleland WH, Mendelson CR, Simpson ER: Effects of aging and obesity on aromatase activity of human adipose cells. *J Clin Endocrinol Metab* 1985; 60:174–177.
210. Feldman A: Glucocorticoid receptors in adipose tissue. *Endocrinol* 1977; 100:398.
211. Lindberg O: *Brown Adipose Tissue*. New York, American Elsevier Publishing Co, 1970.
212. Himms-Hagen J: Brown adipose tissue as an energy buffer: A role in energy balance and obesity. *J Can Diet Assoc* 1983; 44:36–48.
213. Dawkins M, Hull D: The production of heat by fat. *Sci Amer* 1965; 213:62–67.

214. Derry DM, Schonbaum E, Steiner G: Two sympathetic nerve supplies to brown adipose tissue of the rat. *Can J Physiol Pharmacol* 1969; 47:57-63.
215. Nicholls DG, Locke RM: Thermogenic mechanisms in brown fat. *Physiol Rev* 1984; 64:1-64.
216. Lean MEJ, James WPT: Uncoupling protein in human brown adipose tissue mitochondria. *Febs Ltrs* 1983; 163:235-240.
217. Heaton GM, Wagenvoord RJ, Kemp A, et al: Brown adipose tissue mitochondria: Photoaffinity labeling of the regulatory site for energy dissipation. *Eur J Biochem* 1978; 82:515-522.
218. Hansen ES, Nedergaard J, Cannon B, et al: Enzyme-linked immunosorbent assay (ELISA) studies of the interaction between mammalian and avian anti-thermogenin antibodies and brown adipose tissue mitochondria from different species. *Comp Biochem Physiol* 1981; 798:441-449.
219. Nicholls DG, Snelling RS, Rial E: Proton and calcium circuits across the mitochondrial inner membrane. *Biochem Soc Trans* 1984; 12:388-392.
220. Nicholls DG, Bernson VSM: Inter-relationships between proton-electrochemical gradient, adenine nucleotide phosphorylation potential, and respiration during substrate-level and oxidative phosphorylation by mitochondria from brown adipose of cold-adapted guinea-pigs. *Eur J Biochem* 1977; 75:601-612.
221. Himms-Hagen J, Triandafillou J, Gwilliam C: Brown adipose tissue of cafeteria-fed rats. *Am J Physiol* 1981; 241:E116-121.
222. Cannon B, Johansson BW: Nonshivering thermogenesis in the newborn, in Baum H, Gergeley J (eds): *Molecular Aspects of Medicine*, vol 3. Oxford, Pergamon Press, 1980, pp 119-223.
223. Himms-Hagen J: Thermogenesis in brown adipose tissue as an energy buffer. *N Engl J Med* 1984; 31:1549-1558.
224. Leibel RL, Berry EM, Hirsch J: In vivo evidence for catechol-responsive brown adipose tissue in obese patients (abstract). *Proceedings of the Fifth International Congress on Obesity*, 1987.
225. Trayhurn P, Nicholls G (eds): *Brown Adipose Tissue*. London, Edward Arnold Ltd, 1986.
226. Krauss RM, Herbert PN, Levy RI, et al: Further observations on the activities and inhibitions of lipoprotein lipase by apolipoproteins. *Circ Res* 1973; 33:403-411.
227. Abumrad NA, Park JH, Park CR: Permeation of long-chain fatty acid into adipocytes: Kinetics, specificity, and evidence for involvement of a membrane protein. *J Biol Chem* 1984; 259:8945-8949.
228. Kather H, Bieger W, Michel G, et al: Human fat cell lipolysis is primarily regulated by inhibitory modulators acting through distinct mechanisms. *J Clin Invest* 1985; 76:1559-1565.
229. Olivecrona T, Bengtsson G: Lipoprotein lipase, in Angel A, Hollenberg CH, Roncari DAK (eds): *The Adipocyte and Obesity: Cellular and Molecular Mechanisms*. New York, Raven Press, 1983, pp 117-126.
230. Robinson DS, Parkin SM, Speake BK, et al: Hormonal control of rat adipose tissue lipoprotein lipase activity, in Angel A, Hollenberg CH, Roncari DAK (eds): *The Adipocyte and Obesity: Cellular and Molecular Mechanisms*. New York, Raven Press, 1983, pp 127-148.
231. Fain JN, Garcia-Sainz JA: Adrenergic regulation of adipocyte metabolism. *J Lipid Res* 1983; 24:945-966.

232. Pykalisto OJ, Smith PH, Brunzell JD: Determinants of human adipose tissue lipoprotein lipase. *J Clin Invest* 1975; 56:1108–1117.
233. Smith U: Adrenergic control of human adipose tissue lipolysis. *Eur J Clin Invest* 1980; 10:343–344.
234. Burns TW, Langley PE, Terry BE, et al: Pharmacological characterization of adrenergic receptors in human adipocytes. *J Clin Invest* 1981; 67: 467–475.
235. Silverberg AB, Shah SD, Hammond MW, et al: Norepinephrine: Hormone and neurotransmitter in man. *Am J Physiol* 1978; 234:E252–256.
236. Berlan M, LaFontan M: Evidence that epinephrine preferentially as an antilipolytic agent in abdominal human subcutaneous fat cells: Assessment by analysis of beta and alpha-2 adrenoreceptor properties. *Eur J Clin Invest* 1985; 15:341–348.
237. Osterman AJ, Arner P, Engfeldt P, et al: Regional differences in control of lipolysis in human adipose tissue. *Metabolism* 1979; 28:1198.
238. Lafontan M, Dangtran L, Berlan M: Alpha-adrenergic anti-lipolytic effect of adrenaline in human fat cells of the thigh, comparison with adrenaline responsiveness of different fat deposits. *Eur J Clin Invest* 1979; 9:261.
239. Leibel RL, Hirsch J: Site- and sex-related differences in adrenoreceptor status of human adipose tissue. *J Clin Endocrinol Metab* 1987; 64:1205–1210.
240. Ohisalo JJ: Effects of adenosine on lipolysis in human subcutaneous fat cells. *J Clin Endocrinol Metab* 1981; 52:359–363.
241. Fain JN, Malbon CC: Regulation of adenylate cyclase by adenosine. *Mol Cell Biochem* 1979; 25:143–169.
242. Taniguchi A, Kono T, Okuda H, et al: Neutral glyceride synthesis from glucose in human adipose tissue: Comparison between growing and mature subjects. *J Lipid Res* 1986; 29:925–929.
243. Marcus C, Karpe B, Bolme P, et al: Changes in catecholamine-induced lipolysis in isolated human fat cells during the first year of life. *J Clin Invest* 1987; 79:1812–1818.
244. Leibel RL: A biologic radar system for the assessment of body mass: The model of a geometry sensitive endocrine system is presented. *J Theor Biol* 1977; 66:297–306.
245. James WPT, Trayhurn P: Thermogenesis and obesity. *Br Med Bull* 1981; 37:43–48.
246. Astrup A, Bulow J, Christensen NJ, et al: Ephedrine-induced thermogenesis in man: No role for interscapular brown adipose tissue. *Clin Sci* 1984; 66:179–186.
247. Faust IM: Role of the fat cell in energy balance physiology, in Stunkard AJ, Stellar E (eds): *Eating and Its Disorder*. New York, Raven Press, 1984, pp 97–107.
248. Faust IM: Signals from adipose tissue, in Cioffi A, James WPT, Van Itallie TB (eds): *The Body Weight Regulatory System: Normal and Disturbed Mechanisms*. New York, Raven Press, 1981, pp 39–43.
249. Faust IM, Johnson PR, Hirsch J: Surgical removal of adipose tissue alters feeding behavior and the development of obesity in rats. *Science* 1977; 197:393–396.
250. Bjorntorp P, Carlgren G, Isaksson B, et al: Effect of an energy reduced dietary regimen in relation to adipose tissue cellularity in obese women. *Am J Clin Nutr* 1975; 28:445–452.

251. Stunkard A, Mendelson M: Obesity and the body image: I. Characteristics of disturbances in the body image of some obese persons. *Am J Psychiat* 1967; 123:1296–1300.
252. Stunkard AJ, Burt V: Obesity and the body image: II. Age at onset of disturbances in the body image. *Am J Psychiat* 1967; 123:1443–1447.
253. Dwyer J, Mayer J: The dismal condition: Problems faced by obese adolescent girls in American society, in Bray G (ed): *Obesity in Perspective*. Washington, DC, DHEW Publication No 75-708, 1975.
254. Werkman SL, Greenberg ES: Personality and interest patterns in obese girls. *Psychosom Med* 1967; 29:72–80.
255. Wadden TA, Foster GD, Brownell KD, et al: Self-concept in obese and normal weight children. *J Consult Clin Psychol* 1984; 52:1104–1105.
256. Sallade J: A comparison of psychological adjustment of obese vs. nonobese children. *J Psychosom Res* 1973; 17:89–96.
257. Kaplan KM, Wadden TA: Childhood obesity and self-esteem. *J Pediatr* 1986; 109:367–370.
258. Stewart AL, Brook RH: Effects of being overweight. *Am J Pub Health* 1983; 73:171–178.
259. Staffieri JR: A study of social stereotype of body image in children. *J Pers Soc Psychol* 1969; 10:337–343.
260. Richardson SA, Boodman N, Hastorf AH, et al: Cultural uniformity in reaction to physical disabilities. *Sociol Rev* 1961; 26:241–247.
261. Lerner RM, Gellert E: Body build identification, preference, and aversion in children. *Dev Psychol* 1969; 5:456–462.
262. Pugliese MT, Lifshitz F, Grad G, et al: Fear of obesity. *N Engl J Med* 1983; 309:513–518.
263. Huenemann RH, Shapiro LR, Hampton MC, et al: A longitudinal study of gross body composition and body conformation and their association with food and activity in a teen-age population. *Am J Clin Nutr* 1966; 18:324–338.
264. Clifford E: Body satisfaction in adolescence. *Percept Mot Skills* 1971; 33:119–125.
265. Van Itallie TB: Health implications of overweight and obesity in the United States. *Ann Int Med* 1985; 103:983–988.
266. Cornoni-Huntley J, Barbano HE, Brody JA, et al: National health and nutrition examination I-epidemiologic followup survey. *Public Health Rep* 1983; pp 245–251.
267. DeFronzo RA, Cook RA, Andres R, et al: The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. *J Clin Invest* 1975; 55:845–850.
268. Krotkiewski M, Madroukas M, Sjostrum L, et al: Effects of long-term physical training on body fat, metabolism, and blood pressure in obesity. *Metabolism* 1979; 28:649–655.
269. Landsberg L, Young JB: Fasting, refeeding, and the regulation of the sympathetic nervous system. *N Engl J Med* 1978; 298:1295–1301.
270. James WPT, Haraldsdottir J, Liddel F, et al: Autonomic responsiveness in obesity with and without hypertension. *Int J Obes* 1981; 5:73–78.
271. de Divitis O, Fazio S, Pettito M, et al: Obesity and cardiac function. *Circulation* 1981; 64:477–482.
272. Amad KH, Brennan JC, Alexander JK: The cardiac pathology of chronic exogenous obesity. *Circulation* 1965; 32:740–745.

273. Reisin E, Frohlich ED: Obesity. Cardiovascular and respiratory pathophysiologic alterations. *Ann Int Med* 1981; 141:431–434.
274. Clarke WR, Schrott HG, Leaverton PC, et al: Tracking of blood lipids and blood pressure in school age children: The Muscatine study. *Circulation* 1978; 58:626.
275. Lew EA: Mortality and weight: Insured lives and the American cancer society studies. *Ann Int Med* 1985; 103:1024–1029.
276. Feinleib M: Epidemiology of obesity in relation to health hazards. *Ann Int Med* 1985; 103:1019–1024.
277. Barrett-Connor EL: Obesity, atherosclerosis, and coronary artery disease. *Ann Int Med* 1985; 103:1010–1019.
278. Garrison RJ, Castelli WP: Weight and thirty year mortality of men in the Framingham study. *Ann Int Med* 1985; 103:1006–1009.
279. Roberts J, Rowland M: Hypertension in adults 25–74 years of age, DHHS publication no. (PHS) 81-1671. Washington, DC, National Center for Health Statistics, 1981.
280. Goldin RH, McAdam L, Louie JS, et al: Clinical and radiologic survey of the incidence of osteoarthritis among obese patients. *Ann Rheum Dis* 1976; 35:349–353.
281. Engel A: Osteoarthritis and body measurements. *Vital Health Stat* 1968; 11:1–37.
282. Bray GA: Complications of obesity. *Ann Int Med* 1985; 103:1052–1062.
283. Somerville SM, Rona RJ, Chinn S: Obesity and respiratory symptoms in primary school. *Arch Dis Child* 1984; 59:940–944.
284. Angel A, Roncari DAK: Medical complications of obesity. *Can Med J* 1978; 119:1408.
285. Simic BS: Childhood obesity as a risk factor in adulthood, in Collipp PJ (ed): *Childhood Obesity*. Acton, Mass, PSG Publishing Co, 1980, pp 3–24.
286. Taitz LS: *The Obese Child*. Oxford, Blackwell Scientific Publications, 1983, pp 164–165.
287. Douglas FG, Chang PY: Influence of obesity on peripheral airways patency. *J Appl Physiol* 1972; 33:563.
288. Tucker DH, Sieker HO: Volumes and intrapulmonary gas mixing in patients with obesity. *J Clin Invest* 1960; 46:475–481.
289. Holley HS, Milic-Emili J: Regional distribution, pulmonary ventilation and perfusion in obesity. *J Clin Invest* 1967; 46:475–481.
290. Luce JM: Respiratory complications of obesity. *Chest* 1980; 78:626–631.
291. Lourenco RV: Diaphragm activity in obesity. *J Clin Invest* 1969; 48:1609–1614.
292. Rochester DF, Enson Y: Current concepts in the pathogenesis of the obesity-hypoventilation syndrome: Mechanical and circulatory factors. *Am J Med* 1974; 57:402–420.
293. Sharp JT, Barrocas M, Chokroverty S: The cardiorespiratory effects of obesity. *Clin Chest Med* 1980; 1:103–118.
294. Court JM, Hill GH, Dunlop M: Hypertension in childhood. *Aust J Pediatr* 1974; 10:295.
295. New MI, Levine LS: Hypertension in childhood and adolescence. *Cardiovasc Rev Rep* 1982; 3:115–122.
296. Frerichs RR, Webber LS, Srinivasan SR, et al: Relation of serum lipids and lipoproteins to obesity and sexual maturity in white and black children. *Am J Epidemiol* 1978; 108:486–496.

297. Frerichs RR, Webber LS, Voors AW, et al: Cardiovascular disease risk factor variables in children at two successive years: The Bogalusa heart study. *J Chron Dis* 1979; 32:251–262.
298. Richards GE, Cavallo A, Meyer WJ, et al: Hyperandrogenemia: Pediatric perspective and natural history. *J Pediatr* 1985; 107:893–897.
299. Kahn CR, Flier JS, Bar RS: The syndromes of insulin resistance and acanthosis nigricans: Insulin receptor disorders in man. *N Engl J Med* 1976; 294:739–745.
300. Behrman RE (ed): *Nelson Textbook of Pediatrics*, ed 13. Philadelphia, WB Saunders, 1987.
301. Kelsey JL, Acheson RM, Keggi KJ: The body build of patients with slipped capital femoral epiphyses. *Am J Dis Child* 1972; 124:276–281.
302. Dietz WH, Gross WL, Kirkpatrick JA: Blount's disease (tibia vara): Another skeletal disorder associated with childhood obesity. *J Pediatr* 1972; 101:735–739.
303. Chandra RK, Kutty KM: Immunocompetence in obesity. *Acta Paediatr Scand* 1980; 69:25–30.
304. Kolterman O, Insel J, Saekow M, et al: Mechanisms of insulin resistance in human obesity: Evidence for receptor and post-receptor defects. *J Clin Invest* 1980; 65:1272–1284.
305. Krishnan EC, Trost L, Aarons S, et al: Study of function and maturation of monocytes in morbidly obese individuals. *J Surg Res* 1982; 33:89–97.
306. Larsson B, Seidell J, Svarsudd K, et al: Obesity, adipose tissue distribution and health in men: The study of men born in 1913, submitted for publication.
307. Bradley PJ: Is obesity an advantageous adaptation? *J Lipid Res* 1982; 6:43–52.
308. Fitzgerald FT: The problem of obesity. *Ann Rev Med* 1981; 32:221–231.
309. Buck CW: The persistence of elevated blood pressure first observed at age 5. *J Chron Dis* 1977; 26:101.
310. Rosner B, Hennekens CH, Kass EH, et al: Age specific correlation of longitudinal blood pressure data. *Am J Epidemiol* 1977; 106:306–314.
311. Hubert HB, Feinleib M, McNamara PM, et al: Obesity is an independent risk factor for cardiovascular disease: A 26 year follow-up of participants in the Framingham heart study. *Circulation* 1983; 67:968–977.
312. Vague J: The degree of masculine differentiation of obesities: A factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am J Clin Nutr* 1956; 4:20–34.
313. Krotkiewski M, Bjorntorp P, Sjostrom L, et al: Impact of obesity on metabolism in men and women. *J Clin Invest* 1983; 72:1150–1162.
314. Kissebah AH, Vydellingum N, Murray R, et al: Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab* 1982; 54:254–260.
315. Larsson B, Svarsudd K, Welin L, et al: Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13-year follow-up of participants in the study of men born in 1913. *Br Med J* 1984; 288:1401–1404.
316. Lapidus L, Bengtsson C, Larsson B, et al: Distribution of adipose tissue and risk of cardiovascular disease and death: A 12-year follow up of participants in the population study of women in Gothenburg, Sweden. *Br Med J* 1984; 289:1257–1261.

317. Shivley CA, Clarkson TB, Miller LC, et al: Body fat distribution as a risk factor for coronary artery atherosclerosis in female cynomolgus monkeys. *Arteriosclerosis* 1987; 7:226-231.
318. Kalkhoff RK, Hartz AH, Rupley D, et al: Relation of body fat distribution to blood pressure, carbohydrate tolerance, and plasma lipids in healthy obese women. *J Lab Clin Med* 1983; 102:621-627.
319. Zack PM, Harlan WR, Leaverton PE, et al: A longitudinal study of body fatness in childhood and adolescence. *J Pediatr* 1979; 95:126-130.
320. Fox LJ, Brook CGD: Influence of body fatness in childhood on fatness in adult life. *Br Med J* 1979; 1:151-152.
321. Charney E, Goodman HC, McBride M, et al: Childhood antecedents of adult obesity: Do chubby infants become obese adults? *N Engl J Med* 1976; 295:6-9.
322. Abraham S, Collins G, Nordsieck M: Relationship of childhood weight status to morbidity in adults. *HSMHA Health Rep* 1971; 86:273-284.
323. Carnelutti M, Guercio MJ Del, Chiumello G: Influence of growth hormone on the pathogenesis of obesity in children. *J Pediatr* 1970; 77:285.
324. Josefsberg Z, Kauli R, Keret R, et al: Growth hormone response to insulin tolerance test and arginine stimulation in children and adolescents, in Laron Z, Dickerson Z (eds): *The Adipose Child*. Basel, Karger, 1975, p 146.
325. Quabbe JH, Helge H, Kubicki S: Nocturnal growth hormone secretion: Correlation with sleeping EEG in adults and pattern in children and adolescents with nonpituitary dwarfism, overgrowth, and obesity. *Acta Endocrinol* 1971; 67:767-783.
326. Laurian L, Oberman Z, Ayalon D: Under-responsiveness of growth hormone secretion after L-dopa and deep sleep stimulation in obese subjects. *Isr J Med Sci* 1975; 11:482-487.
327. Williams T, Berelowitz M, Joffe SN, et al: Impaired growth hormone responses to growth hormone-releasing factor in obesity. A pituitary defect reversed with weight reduction. *N Engl J Med* 1984; 311:1403-1407.
328. Davies RR, Turner SJ, Cook D, et al: The response of obese subjects to continuous infusion of human pancreatic growth hormone-releasing factor 1-44. *Clin Endocrinol* 1985; 23:521-525.
329. Glass AR, Burman KD, Dahms WT, et al: Endocrine function in human obesity. *Metabolism* 1981; 30:89-104.
330. Forbes BG: Nutrition and growth. *J Pediatr* 1977; 91:40-42.
331. Bray GA: Calorigenic effect of human growth hormone in obesity. *J Clin Endocrinol* 1969; 29:119-122.
332. Mautalen C, Smith RW: Lipolytic effects of human growth hormone in resistant obesity. *J Clin Endocrinol* 1969; 29:119-122.
333. Sims EAH, Horton ES: Endocrine and metabolic adaptation to obesity and starvation. *Am J Clin Nutr* 1968; 21:1455-1470.
334. El-Khodary A, Ball MF, Oweiss IM, et al: Insulin secretion and body composition in obesity. *Metabolism* 1972; 21:641-655.
335. Crockford PM, Salmon PA: Hormones and obesity: Changes in insulin and growth hormone secretion following surgically induced weight loss. *Can Med Assoc J* 1970; 103:147-150.
336. Kalkhoff R, Kim H, Cerletty J: Metabolic effects of weight loss in obese subjects. *Diabetes* 1971; 20:83-91.
337. Kalkhoff R, Ferrow C: Metabolic differences between obese overweight and muscular overweight men. *N Engl J Med* 1971; 284:1236-1239.

338. Barbarino A, DeMarinis L, Troncone L: Growth hormone response to propranolol and L-dopa in obese subjects. *Metabolism* 1978; 27:275-278.
339. Londono JH, Gallagher TF, Bray GA: Effect of weight reduction, triiodothyronine, and diethylstilbestrol on growth hormone in obesity. *Metabolism* 1969; 18:986-992.
340. Nakamoto JM, Gertner JM, Press CM, et al: Suppression of the growth hormone (GH) response to clonidine and GH-releasing hormone by exogenous GH. *J Clin Endocrinol Metab* 1986; 62:822-834.
341. Lucke C, Adelman N, Glick SM: The effect of elevated free fatty acids on the sleep induced growth hormone peak. *J Clin Endocrinol Metab* 1982; 35:407-412.
342. Imaki T, Shibasaki T, Shizume K, et al: The effect of free fatty acids on growth hormone (GH)-releasing hormone-mediated GH secretion in man. *J Clin Endocrinol Metab* 1985; 60:290.
343. Quabbe HJ, Bratzke HJ, Siegers U, et al: Studies on the relationship between plasma free fatty acids and growth hormone secretion in man. *J Clin Invest* 1972; 5:2388-2397.
344. Fineberg SE, Horland AA, Merimee TJ: Free fatty acid concentration and growth hormone secretion in man. *Metabolism* 1972; 21:491-499.
345. Hicks BH, Taylor CI, Vij SK: Effects of changes in plasma levels of free fatty acids on plasma glucagon, insulin, and growth hormone in man. *Metabolism* 1977; 26:1011-1023.
346. Opie LH, Walfish PG: Plasma free fatty acid concentration in obesity. *N Engl J Med* 1963; 186:757-760.
347. Irie M, Tsushima T, Sakuma M: Effect of nicotinic acid administration on plasma HGH, FFA and glucose in obese subjects and in hypopituitary patients. *Metabolism* 1970; 19:972-979.
348. Rosenbaum M, Fong Y, Hesse D, et al: Growth hormone (GH) acutely inhibits pituitary response to GRH via lipolytic products (abstract). 4th Annual Meeting of the North American Association for the Study of Obesity, Boston, MA, October, 1987. *Int J Obesity* 1987; 11:426A.
349. Rosenbaum M, Loche S, Balsano S, et al: Short-term met-hGH infusion inhibits somatotroph response to growth hormone releasing hormone (1-44). *Metabolism* 1988; 37:131-135.
350. Daughaday WH, Phillips LS, Mueller MC: The effects of insulin and growth hormone on the release of somatomedin by the isolated rat liver. *Endocrinology* 1976; 98:1214-1219.
351. Binet E, Schlumberger A, Chaussain JL: Serum somatomedin activity in obese children. *Pediatr Adolesc Endocrinol* 1976; 1:153-156.
352. Phillips LS, Vassilopoulou-Sellin R: Somatomedins. *N Engl J Med* 1980; 302:371-380, 438-446.
353. Hintz RL, Suskind R, Amatayakul K: Growth hormone, insulin, and prolactin secretion in anorexia nervosa and obesity during bromocryptine treatment. *Br Med J* 1977; 2:156-159.
354. Tannenbaum GS, Guyda HJ, Posner B: Insulin-like growth factors: A role in growth hormone negative feedback and body weight regulation via brain. *Science* 1983; 220:77-79.
355. Berelowitz M, Szabo M, Frohman LA, et al: Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and pituitary. *Science* 1981; 212:1279-1281.
356. Ceda GP, Hoffman AR, Silverberg GD, et al: Regulation of growth hormone

- release from cultured human pituitary adenomas by somatomedins and insulin. *J Clin Endocrinol Metab* 1985; 60:1204–1212.
357. Clemons RD, Costin G, Kogut MD: Laron dwarfism: Growth and immunoreactive insulin following treatment with human growth hormone. *J Pediatr* 1976; 88:427–433.
358. Melmed S: Insulin suppresses rat growth hormone messenger ribonucleic acid levels in rat pituitary tumor cells. *Diabetes* 1985; 34:409.
359. Melmed S: Insulin suppressed growth hormone secretion by rat pituitary cells. *J Clin Invest* 1984; 73:1425–1433.
360. Bonnet F, Lodeweyckx MV, Eeckels R, et al: Subcutaneous adipose tissue and lipids in blood in growth hormone deficiency before and after treatment with human growth hormone. *Pediatr Res* 1974; 8:800–805.
361. Avruskin RW, Pillai S, Kasi K, et al: Decreased prolactin secretion in childhood obesity. *J Pediatr* 1985; 105:373.
362. Genazzani AR, Pintor C, Corda R: Adrenal and gonadal steroids in obese prepubertal girls. *J Clin Endocrinol Metab* 1978; 47:974–979.
363. Cacciari E, Frejaviile E, Balsamo A, et al: Disordered prolactin secretion in the obese child and adolescent. *Arch Dis Child* 1981; 56:386.
364. Cavagnini F, Maraschini C, Pinto M: Prolactin secretion in obese patients. *Lancet* 1979; 2:1020.
365. Kopelman PG, Pilkington TRE, White N, et al: Impaired hypothalamic controls of prolactin secretion in massive obesity. *Lancet* 1979; 1:747.
366. Kolesnick RN, Musacchio I, Thaw C, et al: Arachidonic acid mobilizes calcium and stimulates prolactin secretion from GH3 cells. *Am J Physiol* 1984; 246:E458–E562.
367. Kobberling J, Von zur Muhlen A: The circadian rhythm of free cortisol determined by urine sampling at two-hour intervals in normal subjects and in patients with severe obesity or Cushing's syndrome. *J Clin Endocrinol Metab* 1974; 38:313–319.
368. Slavnov VN, Epshtein EV: Somatotrophic, thyrotrophic, and adrenocorticotrophic functions of the anterior pituitary in obesity. *Endocrinologie* 1977; 15:213–218.
369. Streeten DHP, Stevenson GT, Dalakos TG: The diagnosis of hypercortisolism: Biochemical criteria differentiating patients from lean and obese normal subjects and from females on oral contraceptives. *J Clin Endocrinol Metab* 1969; 29:1191–1211.
370. Cheek DB, Graystone JE, Seamark RF, et al: Urinary steroid metabolites and the overgrowth of lean and fat tissues in obese girls. *Am J Clin Nutr* 1981; 34:1804.
371. Cacciari E, Cicognani A, Pirazzoli P, et al: Effect of obesity on the hypothalamo-pituitary-gonadal function in childhood. *Acta Paediatr Scand* 1977; 66:345.
372. Cleary MP, Shepherd A, Jenks B: Effect of dehydroepiandrosterone on growth in lean and obese Zucker rats. *J Nutr* 1984; 114:1242–1251.
373. Yen T, Allan SA, Pearson DV, et al: Prevention of obesity in avy/a mice by dehydroepiandrosterone. *Lipids* 1977; 12:409.
374. Cleary MP, Seidenstat R, Tannen RH, et al: The effect of dehydroepiandrosterone on adipose tissue cellularity in mice. *Proc Roy Soc Exp Bio Med* 1982; 177:276.
375. Feher T, Halmy L: The production and fate of adrenal DHEA in normal and overweight subjects. *Horm Metab Res* 1975; 6:303.

376. Bird CE, Murphy J, Boroomand K, et al: Dehydroepiandrosterone: Kinetics of metabolism in normal women. *J Clin Endocrinol Metab* 1972; 47:818–825.
377. Hendriks A, Heyns W, Damoor P: Influence of a low calorie diet and fasting on the metabolism of dehydroepiandrosterone sulfate in adult obese subjects. *J Clin Endocrinol* 1968; 28:1529.
378. Lopez SA, Krehl WA: A possible interrelation between glucose-6 phosphate dehydrogenase and dehydroepiandrosterone in obesity. *Lancet* 1967; 1:485.
379. Coleman DL, Schwizer RH, Leiter EH: Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutants and in aged normal mice. *Diabetes* 1984; 33:36.
380. Shepherd A, Cleary MP: Metabolic alterations after dehydroepiandrosterone treatment in Zucker rats. *Am J Physiol* 1984; 246:E123.
381. Schmitt T, Luceman W, McCool C, et al: Unresponsiveness to exogenous TSH in obesity. *Int J Obesity* 1977; 1:185.
382. Bray GA, Fisher DA, Chora J: Relation of thyroid hormone to body weight. *Lancet* 1976; 1:1206.
383. Bray GA, Melvin KEW, Chopra IJ: Effect of triiodothyronine on some metabolic responses of obese patients. *Am J Clin Nutr* 1973; 26:715–721.
384. Burman KD, Latham KR, Djuh YY: Solubilized nuclear thyroid hormone receptors in circulating human mononuclear cells. *J Clin Endocrinol Metab* 1980; 51:106–116.
385. Robson HA, Hall R, Smart GA: A critical evaluation of the tendon reflex measurement as an index of thyroid function. *Postgrad Med J* 1965; 79:518–520.
386. Vondra K, Rath R: Obesity and thyroid function: I. Values of the Achilles tendon reflex. *Endocrinologie* 1973; 62:310–320.
387. Porte D, Girardier L: Neural regulation of insulin secretion in the dog. *J Clin Invest* 1973; 52:210–213.
388. Johnson PR, Stern JS, Greenwood MRC, et al: Adipose tissue hyperplasia and hyperinsulinemia in Zucker obese female rats: A developmental study. *Metabolism* 1978; 27:1941–1954.
389. Frohman LA, Bernardis LL: Effect of hypothalamic stimulation on plasma glucose, insulin, and glucagon levels. *Am J Physiol* 1971; 221:1596–1601.
390. Porte D, Robertson RP: Regulation of insulin secretion by catecholamines, stress, and the sympathetic nervous system. *Fed Proc* 1973; 32:1792–1796.
391. Robertson RP, Porte D: Adrenergic modulation of basal insulin secretion in man. *Diabetes* 1973; 22:1–7.
392. Atkinson RL, Kaiser DL: Effects of calorie restriction and weight loss on glucose and insulin levels in obese humans. *J Am Coll Nutr* 1985; 4:411–419.
393. Howard BV, Klimes I, Vasquez B, et al: The antilipolytic action of insulin in obese subjects with resistance to its glucoregulatory action. *J Clin Endocrinol Metab* 1984; 58:544–548.
394. Archer JA, Gordon PH, Roth J: Defect in insulin binding to receptors in obese man. *J Clin Invest* 1975; 55:166–170.
395. Chiumello G, Del Guercio MJ, Canelutti M, et al: Relationship between obesity, chemical diabetes, and beta pancreatic function in children. *Diabetes* 1969; 18:238–245.
396. Salans LB, Daugherty JW: The effect of insulin upon glucose metabolism by adipose cells of different size. *J Clin Invest* 1971; 50:1399–1406.

397. Bernstein R, Grant N, Kipnis D: Hyperinsulinemia and enlarged adipocytes in patients with endogenous hyperlipoproteinemia without obesity or diabetes mellitus. *Diabetes* 1975; 24:207–212.
398. Stern I, Batchelor B, Hollander N, et al: Adipose cell size and immunoreactive insulin levels in obese and normal weight adults. *Lancet* 1972; 2:948–951.
399. Olefsky J, Reaven GM, Farquhar JW: Effects of weight reduction on obesity: Studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects. *J Clin Invest* 1974; 53:64–76.
400. Glass AR, Bongiovanni R, Boehm TM: Insulin resistance in obesity: Differential effect on glucose and amino acid disposal. *Diabetes* 1980; 29:20A.
401. Howard BN, Savage P, Nagulesparan M: Evidence for marked sensitivity to the antilipolytic action of insulin in obese maturity onset diabetics. *Metabolism* 1979; 28:744–759.
402. Harrison LC, King-Roach AP: Insulin sensitivity of adipose tissue in vitro and the response to exogenous insulin in obese human subjects. *Metabolism* 1976; 25:1095–1101.
403. Olefsky JM: The effects of spontaneous obesity on insulin binding, glucose transport, and oxidation of isolated rat adipocytes. *J Clin Invest* 1976; 57:842–851.
404. Kono T, Barham FW: The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin: Studies with intact and trypsin treated fat cells. *J Biol Chem* 1971; 246:6210–6216.
405. Kahn CR: Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. *Metabolism* 1978; 27:1893–1902.
406. Crettaz M, Jeanrenaud B: Postreceptor alterations in the states of insulin resistance. *Metabolism* 1980; 29:467–473.
407. Gliemann J, Gammeltoft S, Vinten J: Time course of insulin-receptor binding and insulin-induced lipogenesis in isolated rat fat cells. *J Biol Chem* 1975; 250:3368–3374.
408. Czech MO: Cellular basis of insulin insensitivity in large rat adipocytes. *J Clin Invest* 1976; 57:1523–1532.
409. Olefsky JM: Mechanisms of decreased insulin responsiveness of large adipocytes. *Endocrinology* 1977; 100:1169.
410. Richardson DK, Czech MP: Primary role of decreased fatty acid synthesis in insulin resistance of large rat adipocytes. *Am J Physiol* 1978; 234:E182–189.
411. Cushman SW, Zarnowski MJU, Franzusoff AJ: Alterations in glucose metabolism and its stimulation by insulin in isolated adipose cells during the development of genetic obesity in the Zucker fatty rat. *Metabolism* 1978; 27:1930–1940.
412. Kobayasu M, Olefsky JM: Effect of experimental hyperinsulinemia on insulin binding and glucose transport in isolated rat adipocytes. *Am J Physiol* 1978; 235:E53–62.
413. Dunkel L, Sorva R, Voutilainen R: Low levels of sex hormone-binding globulin in obese children. *J Pediatr* 1985; 107:95.
414. Hartz AJ, Barboriak PN, Wong A: The association of obesity with infertility and related menstrual abnormalities in women. *Int J Obes* 1979; 3:57–73.
415. Schneider G, Kirschner MA, Ertel NH, et al: Increased estrogen production in obese man. *J Clin Endocrinol Metab* 1979; 48:633–640.
416. Longcope C, Baker R, Johnston CC Jr: Androgen and estrogen metabolism: Relationship to obesity. *Metabolism* 1986; 35:235–237.

417. Bray GA, York DA: Genetically transmitted obesity in rodents. *Physiol Rev* 1971; 51:598–646.
418. Trayhurn P: The development of obesity in animals: The role of genetic susceptibility. *Clin Endocrinol Metab* 1984; 13:451–474.
419. Dietz WH: Childhood obesity: Susceptibility, cause, and management. *J Pediatr* 1983; 103:676–686.
420. Mueller WA: The genetics of human fatness. *Yearbook Phys Anthropol* 1983; 26:215–230.
421. Borjeson M: The aetiology of obesity in children. *Acta Pediatr Scand* 1976; 65:279–287.
422. Foch TT, McClearn GE: Genetics, body weight, and obesity, in Stunkard AJ(ed): *Obesity*. Philadelphia, WB Saunders Co, 1980, pp 48–71.
423. Brook CGD, Huntley RMC, Slack J: Influence of heredity and environment in determination of skinfold thickness in children. *Br Med J* 1975; 2:719–721.
424. Bouchard C: Inheritance of fat distribution and adipose tissue metabolism, in Vague J, Bjorntorp P, Guy-Grand B, et al (eds): *Metabolic Complications of Human Obesity*. New York, Excerpta Medica, 1985, pp 87–96.
425. Poehlman ET, Despres JP, Marcotte M, et al: Genotype dependency of adaptation in adipose tissue metabolism after short-term overfeeding. *Am J Physiol* 1986; 250:E480–E485.
426. Stunkard AJ, Foch TT, Hrubec Z: A twin study of human obesity. *JAMA* 1986; 256:51–54.
427. Brook CGD: Genetic aspects of obesity. *Postgrad Med J* 1977; 53:93–99.
428. Committee on Nutrition of the Mother and Preschool Child: Fetal and infant nutrition and susceptibility to obesity. *Am J Clin Nutr* 1978; 31:2026–2030.
429. Stunkard AJ, Sorensen TI, Hanis C, et al: An adoption study of human obesity. *N Engl J Med* 1986; 314:193–198.
430. Biron P, Mongeau JG, Bertrand D: Familial resemblance of body weight and weight/height in 374 homes with adopted children. *J Pediatr* 1977; 91:555–558.
431. Dietz WH, Garn SM, Gortmaker SL: Letter. *N Engl J Med* 1986; 315(2):128–129.
432. Garn SM, Clark DC: Trends in fatness and the origins of obesity. *Pediatrics* 1976; 57:443–456.
433. Hartz A, Gieffer E, Rimm AA: Relative importance of the effect of family environment and heredity on obesity. *Ann Hum Genet Lond* 1977; 41:185–193.
434. Apfelbaum M, Fumeron F, Dunica S: Genetic approach of family obesity: Study of HLA antigens in 10 families and 86 unrelated obese subjects. *Bio-medicine* 1980; 33:98–100.
435. Fumeron F, Apfelbaum M: Association between HLA-18 and familial obesity syndrome, letter. *N Engl J Med* 1981; 305:645.
436. Lyons MJ, Faust IM, Hemmes RB, et al: A virally induced obesity syndrome in mice. *Science* 1982; 216:82–85.
437. Knittle J, Hirsch J: Effect of early nutrition on the development of rat epididymal fat pads: Cellularity and metabolism. *J Clin Invest* 1968; 47:2091–2098.
438. Gortmaker SL, Dietz WH, Sobol AM, et al: Increasing pediatric obesity in the United States. *Am J Dis Child* 1987; 141(5):535–541.

439. Hirsch J, Leibel RL: What constitutes a sufficient psychobiologic explanation for obesity? in Stunkard AJ, Stellar E(eds): *Eating and Its Disorders*, pp. 121–130, New York, Raven Press, 1984, pp 121–130.
440. Raman L: Influence of maternal nutritional factors affecting birthweight. *Am J Clin Nutr* 1981; 34:775–783.
441. Udall JN, Harrison GG, Vaucher Y, et al: Interaction of maternal and neonatal obesity. *Pediatrics* 1978; 62:17–21.
442. Whitelaw HEL: Influence of maternal obesity on subcutaneous fat in the newborn. *Br Med J* 1976; 1:985–987.
443. Rosso P: Nutrition and maternal-fetal exchange. *Am J Clin Nutr* 1981; 34:744–755.
444. Whitelaw A: Infant feeding and subcutaneous fat at birth and at one year. *Lancet* 1977; 2:1098–1099.
445. Crenshaw C, Parker RT, Carter B: Diabetes mellitus and pregnancy: A 20 year evaluation of 118 pregnancies managed conservatively. *Obstet Gynecol* 1962; 20:334–341.
446. Mickal A, Begnaud WP, Weese WH: Glucose tolerance and excessively large infants: A twelve year follow-up study. *Am J Obstet Gynecol* 1966; 94:62–64.
447. Ginsberg-Fellner F, Knittle J: Weight reduction in young obese children: I. Effects on adipose tissue cellularity and metabolism. *Pediatr Res* 1981; 15:1381–1389.
448. White P, Koshy P, Duckers J: The management of pregnancy complicating diabetes and of children of diabetic mothers. *Med Clin N Am* 1953; 37:1481–1496.
449. Farquahar JW: The infant of the diabetic mother. *Postgrad Med J* 1969; 45:806–813.
450. Vohr BR, Lipsitt LP, Oh W: Somatic growth of children of diabetic mothers with reference to birth size. *J Pediatr* 1980; 97(2):196–199.
451. Pettit DJ, Baird HR, Allech KA, et al: Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. *N Engl J Med* 1983; 308:242–245.
452. Ravelli GP, Stein Z, Susser MW: Obesity in young man after famine exposure in utero and early infancy. *N Engl J Med* 1976; 295: 349–353.
453. Fomon SJ, Thomas LN, Filer LJ, et al: Food consumption and growth of normal infants fed mild-based formulas. *Acta Paediatr Scand* 1971; 223(suppl):1–36.
454. Fomon SJ, Rogers RR, Ziegler EE, et al: Indices of fatness and serum cholesterol at age eight years in relation to feeding and growth during early infancy. *Pediatr Res* 1984; 18:1233–1238.
455. DeSwiet M, Fayers P, Cooper L: Effect of feeding habit on weight in infancy. *Lancet* 1977; 2:892–894.
456. Dubois S, Hill DE, Beaton GH: An examination of factors believed to be associated with infantile obesity. *Am J Clin Nutr* 1979; 32:1997–2004.
457. Vobecky JS, Vobecky J, Shapcott D, et al: Biochemical indices of nutritional status in maternal, cord, and early neonatal blood. *Am J Clin Nutr* 1982; 36(4):630–642.
458. Enzi G, Inelman EM, Rubaltelli FF, et al: Postnatal development of adipose tissue in normal children on strictly controlled caloric intake. *Metabolism* 1982; 31:1029–1034.

459. Butte CF, Garza C, Smith EO, et al: Human milk intake and growth in exclusively breast fed infants. *J Pediatr* 1984; 104(2):187-195.
460. Dietz WH, Gortmaker SL: Factors within the physical environment associated with childhood obesity. *Am J Clin Nutr* 1984; 39:619-624.
461. Ravelli GP, Belmont L: Obesity in nineteen-year-old men: Family size and birth order associations. *Am J Epidemiol* 1979; 109:66-71.
462. Patterson RE, Typpo JT, Typpo MH, et al: Factors related to obesity in pre-school children. *J Am Diet Assoc* 1986; 86:1376-1381.
463. Dietz WH, Gortmaker SL: Do we fatten our children at the television set? Obesity and television viewing in children and adolescents. *Pediatrics* 1985; 75:807-812.
464. Davis BA, Roncari DAK: Behavioural treatment of obesity. *CMA J* 1978; 119:1423-1425.
465. Wing RR, Jeffrey RW: Outpatient treatment of obesity: A comparison of methodology and clinical results. *Int J Obes* 1979; 3:261-279.
466. Hager A, Sjostrom L, Arvidsson V: Adipose tissue cellularity in obese school girls before and after dietary treatment. *Am J Clin Nutr* 1978; 31:68-75.
467. Lloyd JK, Wolff OH, Whelan WS: Childhood obesity: A long-term study of height and weight. *Br Med J* 1961; 2:145-148.
468. Deaths associated with liquid protein diets. *MMWR* 1977; 26:443.
469. Protein diets. *FDA Drug Bull* 1978; 8:2.
470. Sours HE, Frattali VP, Brand D, et al: Sudden death associated with very low calorie weight reduction regimens. *Am J Clin Nutr* 1981; 34:453-461.
471. Lantigua RA, Amatruda JM, Biddle TL, et al: Cardiac arrhythmias associated with a liquid protein diet for the treatment of obesity. *N Engl J Med* 1980; 303:735-738.
472. Nylander I: The feeling of being fat and dieting in a school population. *Acta Sociologica Scand* 1971; 1:17-26.
473. Mallick HJ: *The Adverse Effects of Weight Control in Teenage Girls*, doctoral dissertation. Case Western Reserve University, 1980.
474. Mallick HJ: Health hazards of obesity and weight control in children: A review of the literature. *Am J Pub Heal* 1983; 73:78-87.
475. Grodin JM: Secondary amenorrhea in the adolescent. *Pediatr Clin N Am* 1972; 19:619-630.
476. Lev-Ran A: Secondary amenorrhea resulting from uncontrolled weight reduction diets. *Fertil Steril* 1974; 25:459-462.
477. Dietz WH, Hartung R: Changes in height velocity of obese preadolescents during weight reduction. *Am J Dis Child* 1985; 139:705-707.
478. Marliss EB: Protein diets for obesity: Metabolic and clinical aspects. *CMA J* 1978; 119:1413-1420.
479. Felig P: Four questions about protein diets. *N Engl J Med* 1978; 298:1025-1028.
480. Dietz WH, Schoeller DA: Optimal dietary therapy for obese adolescents: Comparison of protein plus glucose and protein plus fat. *J Pediatr* 1982; 100:638-644.
481. Pencharz PB, Motil KJ, Parsons HG, et al: The effect of an energy restricted diet on the protein metabolism of obese adolescents, nitrogen-balance, and whole-body nitrogen turnover. *Clin Sci* 1980; 59:13-18.
482. Merritt RJ, Bistrid BR, Blackburn GL, et al: Consequences of modified fasting in obese pediatric and adolescent patients: I. Protein-sparing modified fast. *J Pediatr* 1980; 96:13-19.

- 483. Brownell KD, Stunkard AJ, Albaum JM: Evaluation and modification of exercise patterns in the natural environment. *Am J Psych* 1980; 137:1540–1545.
- 484. Bradfield RB, Paulos J, Grossman L: Energy expenditure and heart rate of obese high school girls. *Am J Clin Nutr* 1971; 24:1482–1486.
- 485. Dietz WH: Nutrition and obesity, in Grand RJ, Sutphen JL, Dietz WH (eds): *Pediatric Nutrition: Theory and Practice*. Stoneham, Mass, Butterworth Publishers, 1987.
- 486. Vigersky RA, Loriaux SL, Andersen AE: Anorexia nervosa: Behavioural and hypothalamic aspects. *Metabolism* 1976; 25:517–535.

Ciliary Defects: Cell Biology and Clinical Perspectives

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The eukaryotic cilium provides a classic example of the fitting of form to function at the cellular and molecular level in the living cell. Although ciliary motility is elegant in its apparent simplicity, both ciliary structure and the mechanochemistry required to accomplish effective motion are complex. This complexity extends from the biochemical organization of the individual axonemal components, to the functional organelle, to its coordination with neighboring cilia. The widespread occurrence of cilia and flagella throughout the phylogenetic tree speaks to the economy of nature in accomplishing two specific cellular functions; either circulating a fluid over a stationary cell surface or, conversely, propelling a motile individual cell through a fluid.

A vast body of knowledge has accumulated relative to the biology of the cilium as well as its significance to human health. Much of this information has come from studies of ciliated or flagellated eukaryotic protists. Additionally, among mammals, including humans, a number of congenital ciliary abnormalities have been identified that dispose them to lifelong respiratory disease and likely infertility. More recently, microbiologic pathogens and certain environmental insults have been identified as a cause of acquired ciliary defects, which, in contrast to inborn errors of ciliary structure and function, appear to be transient manifestations of cellular injury.

This review provides a perspective of the knowledge gained from various basic studies of the development, organization, and function of the normal eukaryotic cilium and relates those observations to clinical experience relative to the appearance of abnormal cilia, their functional consequences, and impact on human health.

The Normal Cilium: An Overview

Cilia or Flagella?

The organization of cilia and flagella and their basic functional mechanisms are similar in all eukaryotes and the distinction between them is arguably a minor one. The ultrastructural homology is virtually complete, the only major difference being that flagella are generally somewhat longer with usually one or two per cell in contrast to the hundreds of cilia that may populate a ciliated cell. The major functional difference in cilia and flagella resides in the pattern of their motion. A rotating spiral is characteristic of flagellar movement as it propels an individual cell through a liquid medium. In contrast, the numerous cilia populating the border of a stationary cell circulate the fluid in which they are bathed by an undulating, whiplash type of motion having little or no rotational component.

Distribution of Ciliated and Flagellated Cells in Mammals

Ciliated epithelia appear in a number of tissues in mammals. Both the upper and lower airways and the mucosa of the middle ear and eustachian tube are lined by a ciliated epithelium.¹⁻³ In males, ciliated epithelium occurs on the ductuli efferentes on the border between the testis and the epididymis.⁴ In females, the endometrial lining of the cervix and oviducts are populated by a lining of ciliated cells.^{5, 6} Ciliated cells also appear in the ependyma of the brain.⁷ At these sites, the primary function of the ciliated cells appears to be the clearance of debris and the transport and circulation of cells and fluid.

Modifications and specializations of cilia are associated with certain sensory organs among mammals. Olfactory cells possess modified cilia having a normal microtubular configuration but without dynein arms.⁸ Also, the hair cells of the vestibular organ each possesses a kinocilium, a single, long, rigid, nonmotile cilium.⁹ The eye most likely represents the highest specialization of the cilium seen in higher vertebrates with inner and outer segments of rod cells being linked by a highly modified cilium.¹⁰ Spermatozoa are the only cells among higher vertebrates possessing true, albeit modified, flagella, their function being the locomotion of the male gamete to the ovum for fertilization.¹⁰

Many reports in the literature concern the anomalous appearance of cilia in various tissues and cell cultures.¹¹⁻¹⁶ These "primary" cilia generally are solitary and appear to be derived differently from the populations of cilia lining various epithelia.¹⁷ They often appear with unusual axonemal configurations, and their motility generally is reduced or absent. These reports suggest that primary cilia may appear in virtually any organ system although their function at those sites remains undetermined.

Ultrastructural Organization of Normal Cilia

The eukaryotic cilium is a distally tapering cylinder of 5 to 50 μm in length (Figs 1 and 2). Its internal organization is based around an arrangement of nine peripheral pairs and one central pair of microtubules. In addition to the microtubular configuration, a number of other morphologically distinctive features contribute to axonemal structure. The role of ciliary microtubules and the dynein arms in ciliary activity has been fairly well characterized; however, the participation of the remaining accessory elements in ciliary motility is not as clear. The axonemal components, their position in the axoneme, and function if known are briefly outlined here.

Microtubules and Tubulin

The 9 + 2 microtubular pattern characteristic of cilia and flagella provides a prominent cytologic landmark and point of reference. Tubulin, the fundamental protein of microtubules, is a dimer, the α - and β -subunits each having a purified molecular weight of approximately 55,000 daltons.¹⁸⁻²⁰ Microtubules are assembled from protofilaments, arrays of these alternating α - and β -monomers organized into a linear pattern with a slight helical pitch. In cilia and flagella, the peripheral microtubular pairs are each organized in a "piggyback" configuration. One microtubule, called tubule "A," has a full complement of 13 protofilaments, whereas its partner tubule "B" has only 10 or 11, in effect sharing some subunits with tubule A. Unlike the peripheral microtubular pairs, the microtubules composing the central pair have no shared subunits.

Dynein

Tubule A of each peripheral ciliary microtubular pair exhibits two projections, the dynein arms, positioned in a clockwise direction when viewed from the interior of the cell outward. Dynein was first isolated and its function as an adenosine triphosphatase (ATPase) described by Gibbons and Rowe.²¹ The outer arm often appears more prominently curved in ultrathin sections. Recent electron microscopic observations of rapidly frozen, deep-etched cilia further confirm this observation.^{22, 23}

Nexin

Connecting the peripheral microtubular pairs is a thin filamentous band composed of a protein, nexin, which appears to link and maintain the configurational integrity of the radial microtubules of the axoneme.²⁴

Radial Spokes

Extending from the tubule A of each peripheral microtubular doublet is a connecting link directed toward the central microtubular sheath. These radial spokes terminate in a knoblike structure adjacent to the central sheath.²⁵ It is thought that the radial spokes provide a stabilizing and guiding structure for the sliding microtubules.

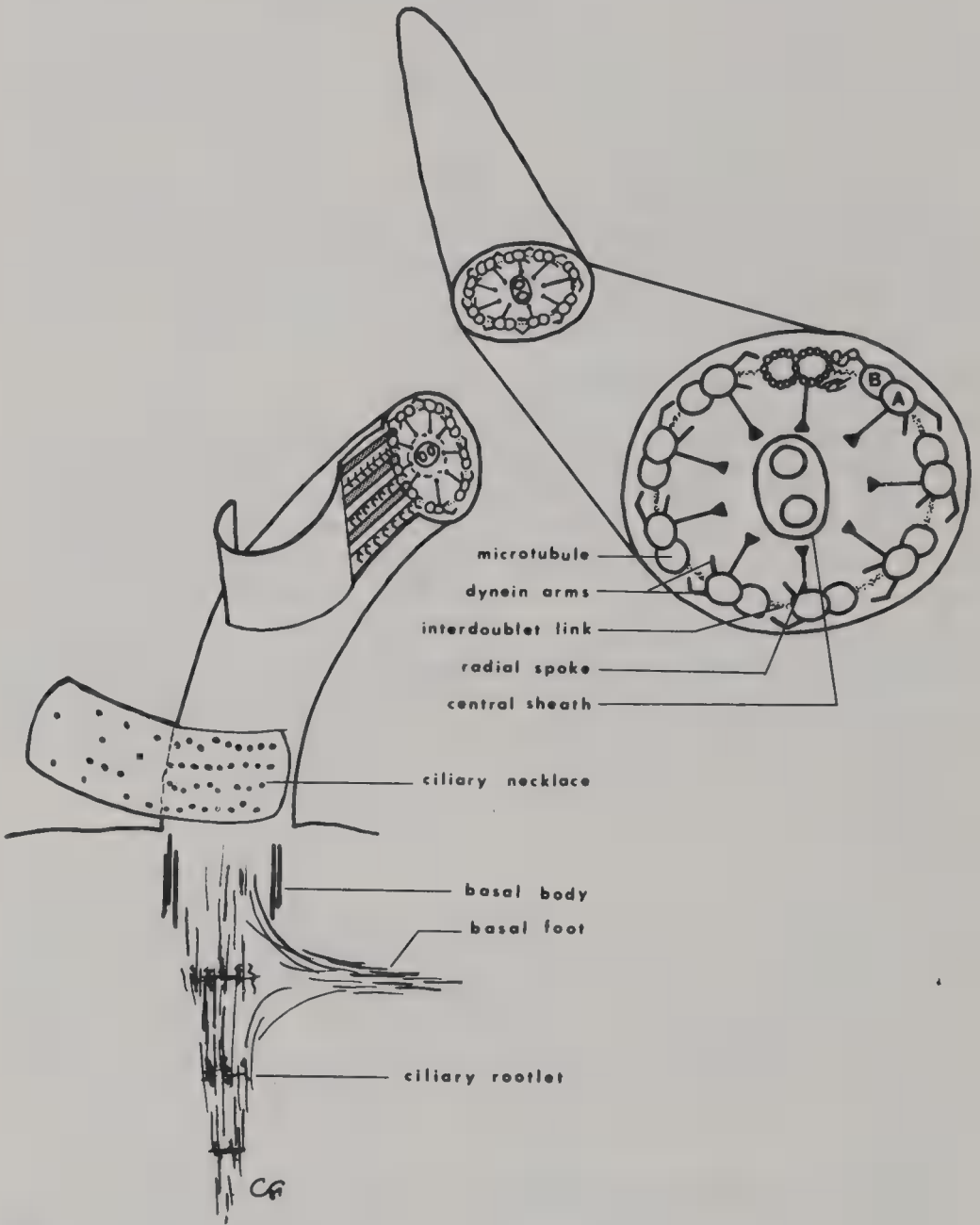


FIG 1.
The eukaryotic cilium illustrating the spatial relationships and positions of major elements.

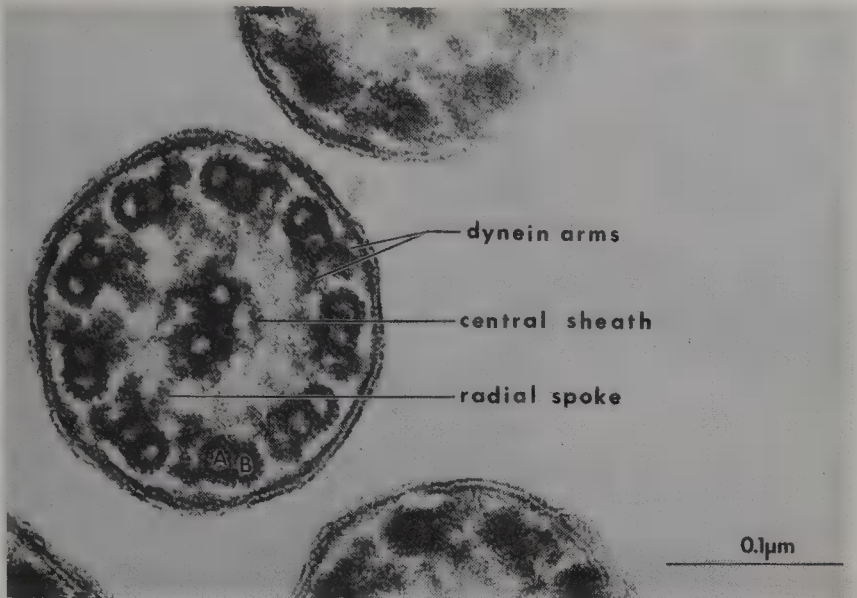


FIG 2.

Transmission electron micrograph of a cilium in cross section from airway epithelium of a normal human subject. The tissue was fixed in an aldehyde solution containing tannic acid to enhance preservation of microtubules. This photomicrograph illustrates several major features including cross sections of microtubular protofilaments, radial spokes, and dynein arms. The designations A and B on the microtubular pair in the six o'clock position are standard conventions used to identify the individual microtubules of the peripheral pairs. The complete "A" tubule exhibits the projecting dynein arms. The "B" tubule is incomplete and joined to the A tubule by several shared subunits. See also Figure 1.

Central Sheath

The two central tubules appear to be bound within a common sheath. The radial spokes and the central sheath are proximal to one another and may have complementary functions in motility.

The Ciliary Membrane

The axonemal components and matrix are surrounded by a trilaminar cell membrane, an extension of the cell membrane. Freeze-fracture preparations reveal that the shaft region of the ciliary membrane is populated by sparse membrane-associated particles. Also, four to six rings of ciliary necklace particles encircling the ciliary shaft reside at the base of each cilium in cells of higher organisms (Fig 3).²⁶

From Form to Function: How Cilia Work

In the cilium, nature has provided an easily observable and quantifiable cellular function (motility) and structures consonant with that function that



FIG 3.

Freeze-fracture preparation of human airway epithelium illustrating a ciliary necklace at the base of a cilium (*black arrow*) and a nascent ciliary necklace (*black/white arrow*) residing on the luminal border of a ciliated cell.

can be experimentally dissected and their basic structure/function relationships studied in the laboratory. Additionally, mutated and injured cilia exhibiting functional deficits appear among humans in the clinical setting also providing a rich opportunity for the study of this organelle in relation to disease pathogenesis.

The ciliary motive force appears to be generated by a sliding of the microtubular pairs relative to one another that is modulated by the dynein arms and further assisted by the axonemal accessory structures. This concept, now known as the "sliding filament hypothesis," was postulated by Afzelius²⁷ and further refined in a number of other classic studies.^{21, 28, 29} The basic dynamics relative to this hypothesis involve the interaction of the dynein arms residing on each tubule A of the peripheral microtubular pairs with the tubule B of the neighboring peripheral pair.³⁰ The "rigor" position of the arms, which can be considered as the starting point in a single mechanochemical cycle, exists in the absence of adenosine triphosphate (ATP). Addition of ATP causes the release and shortening of the arms from the rigor position, a step independent of ATPase activity. The arms then reextend basally at an angle of approximately 40 degrees and reat-

tach to the neighboring microtubular pair, a step requiring hydrolysis of ATP. The arms subsequently return to the equilibrium (rigor) position by the sliding of the doublets relative to one another. To convert the concerted sliding of all the pairs of peripheral microtubules relative to one another to the bending motion characteristic of the cilium, some rigid structure must provide the necessary shear resistance. This requirement may be fulfilled by the radial spokes because of their natural alignment perpendicular to the microtubular pairs and their proximity to the central sheath. A final element relevant to the dynamics of ciliary motility involves the role of the ciliary membrane. Gilula and Satir²⁶ have postulated that this structure may be responsible for the control of local membrane permeability, particularly to calcium ion, which is requisite for ciliary activity.

Ciliogenesis

Ciliogenesis In Lower Eukaryotes

Because it appears that some ciliary defects may be derived from fundamental errors of ciliogenesis, an understanding of this process is an important element in research aimed at achieving a better understanding of the origin of ciliary defects. The molecular basis of ciliogenesis, and particularly of the assembly of axonemal microtubules, has been most extensively studied in eukaryotic protists³¹⁻³⁴ and is likely pertinent to mammalian cells also; however, knowledge concerning the dynamics of assembly of other axonemal components is presently very limited.

In recent years, several studies have indicated that axonemal tubulin is posttranslationally modified in a number of lower organisms by the acetylation of α -tubulin.³⁵⁻³⁷ Monoclonal antibodies generated against this modified α -tubulin react with axonemal tubulin but do not react with cytoplasmic tubulin.³⁸ Thus, acetylation appears to be an important characteristic of axonemal tubulin and may account in part for the stability and other attributes that distinguish cytoplasmic from axonemal microtubules.

Ciliogenesis In Mammals

Three basic approaches have been employed individually and in concert to study ciliogenesis in mammalian airways. These include observations of (1) in utero maturation of fetal animals,¹⁷ (2) organ cultures,³⁹ and (3) regeneration and renewal of ciliated epithelium following injury.⁴⁰

The formation, appearance, frequency, and functional capacity of primary cilia is considerably different from that of the motile cilia populating epithelial borders. Primary cilia appear to be derived directly from a parent centriole through a series of phases leading to the emergence of the single cilium through the cell membrane.¹⁷ In the event the centriole resides

deeply within the cytoplasm, the primary cilium may elongate and remain intracytoplasmic in position. The functional capacity of the primary cilium as a motile organelle appears to be very attenuated relative to the vigorous dynamics of epithelial border cilia.

Although the origin of the cilia composing the ciliary beds lining various epithelia are derived differently from the developmental course of individual primary cilia, a structural and developmental kinship with centrioles is evident. Ciliary bed cilia develop from procentrioles that are products of deuterosomes, structures derived from fibrogranular aggregates residing near a previously existing pair of centrioles. The procentrioles, which are actually nascent basal bodies, organize radially about the deuterosomes. Subsequently, they are released to the cytoplasm and migrate to the luminal border of the cell where they mature as basal bodies, and the assembly, emergence, and elongation of the individual axonemes occurs.

The arrival of the basal bodies at the luminal membrane during early stages of ciliogenesis is heralded by the appearance of arrays of intramembrane particles that represent nascent ciliary necklaces (see Fig 3).⁴¹ These particle arrays further organize, presumably with the maturation of the subjacent basal bodies, into concentric rings or spirals through which the budding cilia emerge. The complexes remain in place at the ciliary bases during axonemal assembly and emergence, suggesting a possible regulatory role in ciliogenesis, and become the ciliary necklaces of the mature cilia.

Ciliary Defects: Congenital Vs. Acquired

Ciliary defects in clinical medicine can be divided into two general classes, congenital and acquired. The heritable nature of congenital human respiratory diseases in which defective cilia are pathognomonic is increasingly well documented as is the definition of the specific ciliary abnormalities associated with them. Afzelius and colleagues^{42, 43} provided some of the first evidence of a genetic component to certain ciliary diseases when they identified two brothers with chronic respiratory disease who also exhibited dysmorphic and functionally compromised sperm flagella. These reports provided a unifying pathophysiologic factor, a genetic predisposition for the expression of defective cilia, as an explanation for the outwardly disparate clinical syndromes of dextrocardia, chronic respiratory disease, and infertility, which often appeared together in some individuals. These landmark studies gave way to many other investigations examining the role of ciliary defects and dysfunction on human health including investigations of other seemingly related clinical syndromes⁴⁴ as well as efforts to elucidate the genetic basis of inborn ciliary disease.^{45, 46} A complicating issue in the identification and characterization of the manifestations of congenital ciliary diseases has been the superimposition of acquired ciliary defects. Acquired ciliary defects appear to arise from cell injury and may result from a variety of sources. The reduction in mucociliary clearance among patients with

inborn congenital ciliary abnormalities is considerable, often leading to infection and the appearance of acquired forms of ciliary defects. Furthermore, there is good evidence that acquired ciliary defects appear transiently among normal individuals during periods of acute infection or exposure of the airway mucosa to certain environmental pollutants and irritants.

It is hoped that the following paragraphs will clarify the confusing nomenclature surrounding certain ciliary diseases and focus on their clinical manifestations and the possible relationships between acquired and congenital ciliary abnormalities.

Congenital Ciliary Diseases

Evolution and Present Status of Nomenclature

A triad of symptoms involving situs inversus, bronchiectasis, and chronic sinusitis was described first by Siewert⁴⁷ in 1904. In 1933, Kartagener further characterized this syndrome, which has come to bear his name.⁴⁸ The diverse presentations associated with this syndrome, situs inversus, chronic sinusitis, and bronchiectasis must have been somewhat enigmatic for clinicians of that era. Over 40 years later with the advent of modern electron microscopy, Afzelius et al.^{42, 43} identified an ultrastructural level lesion of cilia in patients with Kartagener's syndrome (KS). Eliasson further confirmed the observations of Afzelius and a short time later reported with other colleagues that similar ciliary abnormalities may appear in the absence of dextrocardia.⁴⁹ The term immotile cilia syndrome (ICS) was adopted to identify the condition they had observed. The specific defect of cilia involved the absence of the dynein complement of the ciliary axoneme causing the cilia to be "locked" in a state of rigor or immotility (Fig 4). This markedly compromised the functional capacity of the cilia and effectively reduced mucociliary clearance. Subsequent studies^{50, 51} further indicated that the cilia from patients with ICS were not totally immotile but often exhibited an ineffective oscillating or rotating type motion leading to the use of the term "dyskinetic cilia syndrome." A growing confusion in terminology led to a joint proposal introduced by Sleight⁵² for a unifying nomenclature. Thus, the presently accepted term "primary ciliary dyskinesia" (PCD) came to identify patients with or without situs inversus having chronic sinusitis and chronic bronchitis/bronchiectasis and expressing ultrastructurally documented ciliary abnormalities. The scope of this definition thus encompassed KS and conveyed a more accurate sense of the pathophysiologic basis of this condition.

In their pioneering studies, Afzelius and colleagues^{42, 43, 53} not only recognized the genetic basis for PCD but also outlined a plausible mechanism for its origin that explained the differential appearance of situs inversus. They postulated that the ciliary dysfunction characteristic of PCD extends to early embryogenesis at which time the specific direction and beat pat-

**FIG 4.**

Electron micrograph of nasal cilia from a patient with KS (primary ciliary dyskinesia with situs inversus) illustrating the absence of both dynein arms. The alignment of the central microtubular pairs of adjacent cilia also should be parallel rather than at right angles as shown here and marked by the reference arrows inserted in the photomicrograph.

tern of the ciliated epithelium of normal embryos directs the ultimate positioning of the viscera. In embryos with congenital PCD, the failure of the cilia to rotate the viscera properly leads to the final positioning of the viscera by random chance. Thus half of the patients with PCD would be expected not to have malrotation of the viscera, a figure in general agreement with actual observation.

Ultrastructural Manifestations of Primary Ciliary Dyskinesia

A number of studies in recent years have continued to characterize features of PCD relative to clinical medicine as well as elucidating basic mechanisms of its pathophysiology. Among these is a growing awareness of the heterogeneity of expression of ciliary dysmorphology associated with this condition. Schneeberger et al.⁵⁴ have addressed this issue and documented a variety of ciliary abnormalities in addition to the absence of dynein arms (Fig 5). Other studies also have continued to document the various dynein arm alterations in PCD.⁵⁵⁻⁵⁷

Although there is at least one report in the literature of normal ciliary ultrastructure in a patient with KS,⁵⁸ the role of the dynein arms in the pathogenesis of PCD is virtually undeniable (see Fig 4). However, several other features of cilia also appear to be associated consistently with PCD. Sturgess et al.^{59, 60} have reported two such abnormalities, the absence of

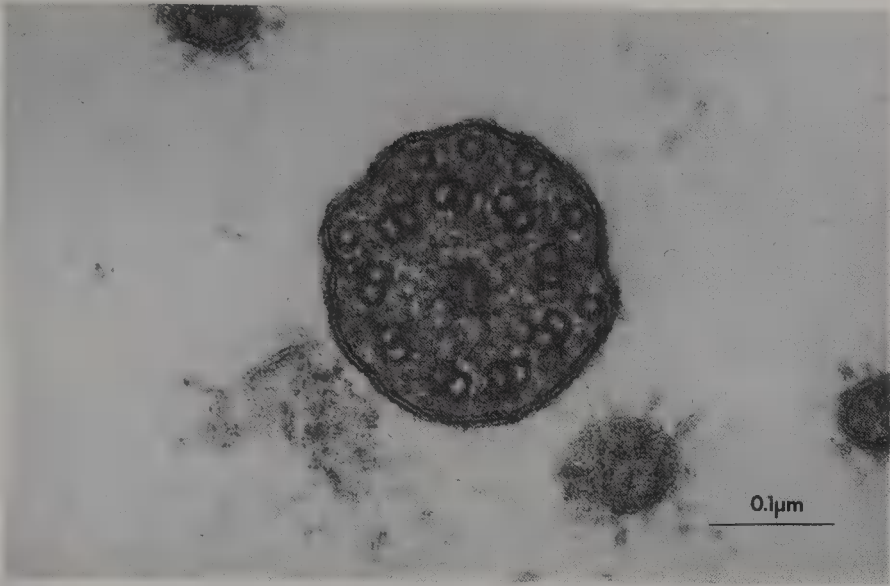


FIG 5.

Electron micrograph of microtubular additions in a patient with PCD. Similar patterns of both microtubular additions and deletions may occur in cilia from patients with other chronic diseases as well among individuals with acute viral upper respiratory infections.

radial spokes (Fig 6) and the transposition of the no. 1 peripheral pair of microtubules to the center of the axoneme (Fig 7). Either of these configurations would result in an axonemal organization inconsistent with adequate function relative to the sliding filament model of ciliary motility. Additionally, Schneeberger et al.⁵⁴ have observed that the ciliary feet or spurs, lateral protrusions of the basal body, are unidirectionally oriented in normal human subjects but randomly oriented in patients with PCD (Figs 8 through 11). These investigators also reported that the alignment of the central pairs of microtubules of adjacent cilia, rather than lying parallel to one another, lie randomly relative to one another among patients with PCD (see Fig 4).

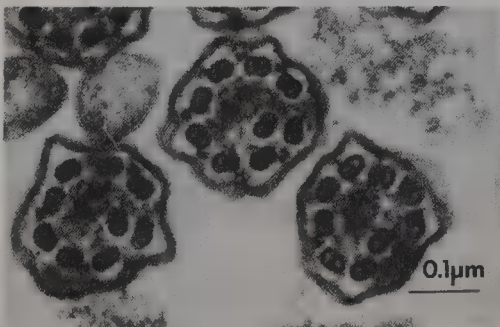
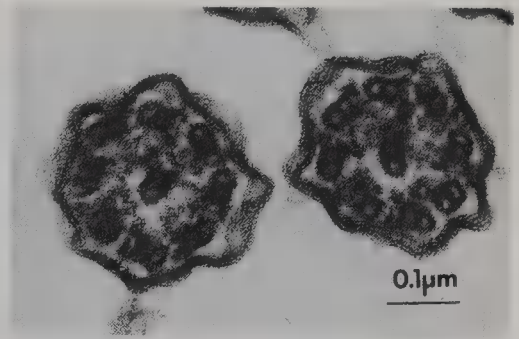


FIG 6.

Electron micrograph illustrating the absence of radial spokes in a patient with PCD. (Courtesy of Drs. J.M. Sturgess and J. Chao.)

FIG 7.
Electron micrograph illustrating transposition of a peripheral microtubular pair to the central position. (Courtesy of Drs. J.M. Sturgess and J. Chao.)



Screening for Primary Ciliary Dyskinesia

Unequivocal diagnosis of primary ciliary dyskinesia requires electron microscopic documentation. However, there are several avenues of investigation to assist the clinician in making a presumptive diagnosis of this condition. First, other major chronic diseases such as cystic fibrosis, allergies, and immune deficiencies must be ruled out. Subsequently, the determination of a lifelong history of chronic sinusitis, bronchitis, and bronchiectasis should alert the physician to the possibility of PCD. Documentation of situs inversus in the patient, a sibling, or close relative is very pertinent given the close relationship of KS to PCD, although one should be mindful that approximately half of the cases of PCD do not exhibit dextrocardia. Other peripheral but related factors of which the physician should be aware include living but immotile spermatozoa among males, inability to

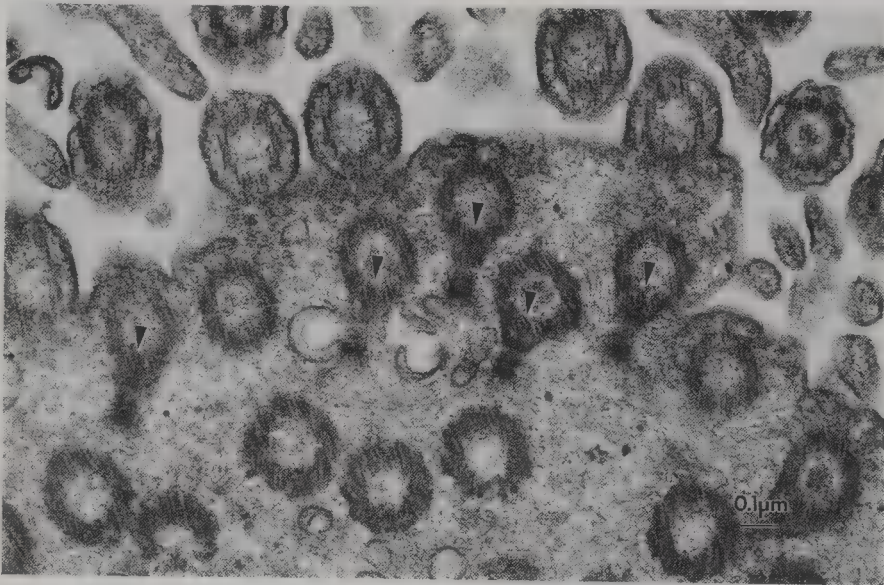


FIG 8.
Cross sections of basal feet (arrows) in nasal epithelium of a normal subject illustrating parallel alignment.

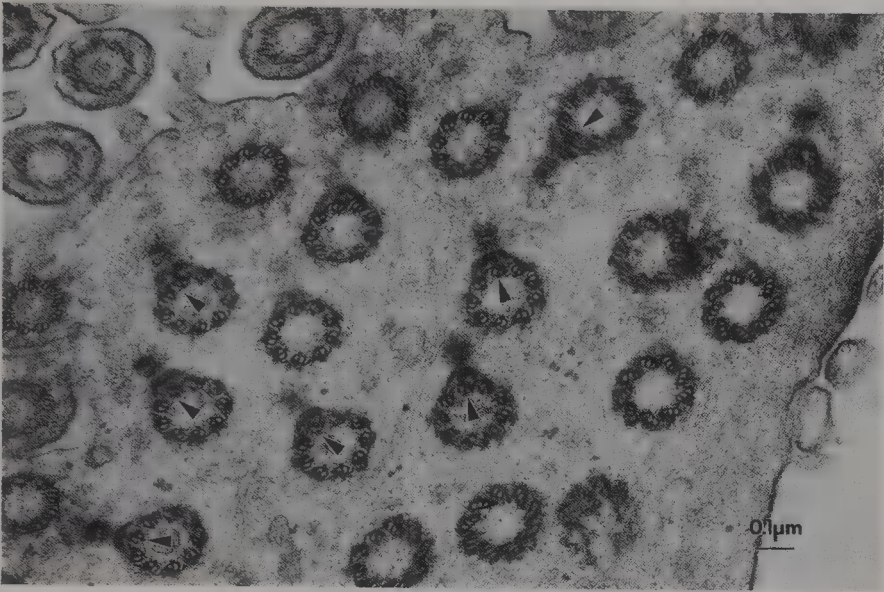


FIG 9.

Cross sections of basal feet (*arrows*) in nasal epithelium of a patient with KS illustrating nonparallel positions reflecting altered beat directions.

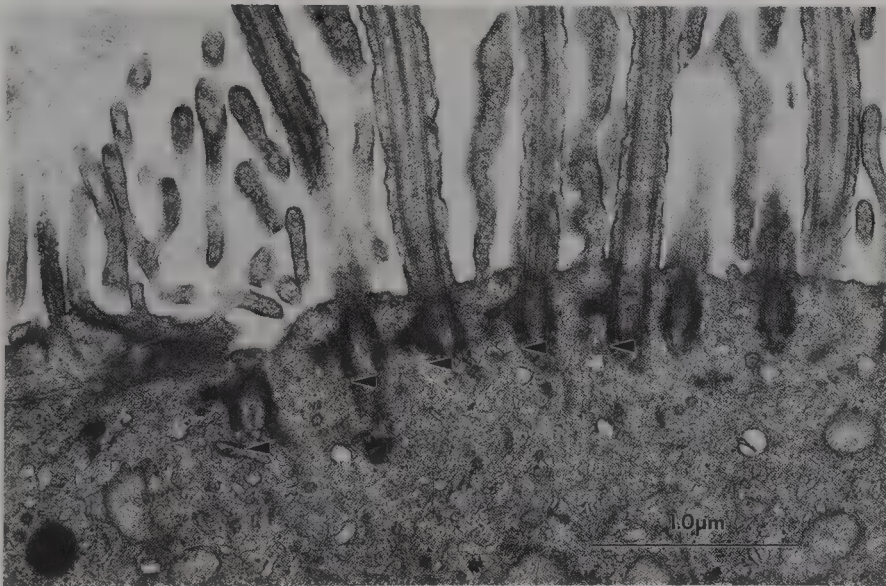
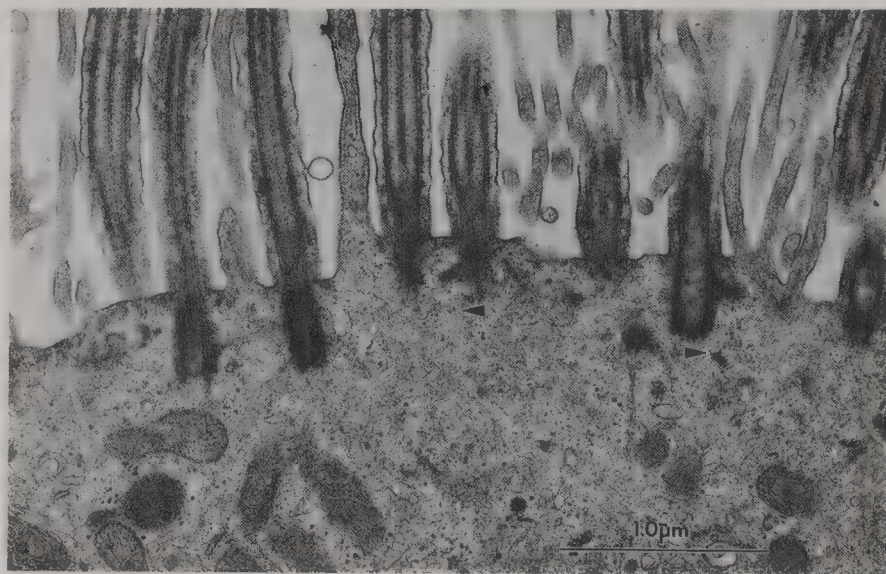


FIG 10.

Longitudinal sections of basal feet (*arrows*) in nasal epithelium of a normal subject illustrating proper mutual alignment.

**FIG 11.**

Longitudinal sections of basal feet in nasal epithelium of a subject with KS illustrating misdirected orientation of basal feet.

conceive among females, headaches, chronic cough, and absent or diminished mucociliary clearance. Given the presumptive diagnosis of PCD based on these findings, the final step to confirm PCD is by the electron microscopic evaluation of ciliary defects characteristic of the condition.

For patients referred to our laboratory, we have found the nasal curettage procedure a useful means for documentation and evaluation of ciliary defects in PCD. While samples of ciliated epithelium or flagellated cells obtained in the course of other procedures can be used for evaluation, the ciliated mucosa lining the nasal turbinates can be retrieved for this purpose in a virtually painless, minimally invasive fashion. This technique has been described using either a cytologic brush or a Freimuth curette^{61, 62} and can be applied with facility in small children and infants as well as adults. We presently use an inexpensive, commercially available, disposable curette for this purpose. Using an otoscope for illumination the curette is gently drawn over the inferior surface of the turbinate under direct vision with no local anesthetics. The tissue obtained can be examined directly by light microscopy using either phase contrast or differential interference contrast (Nomarski) optics for the presence and extent of ciliary activity. Additionally, the mucosa can be fixed for histologic screening and subsequent electron microscopic evaluation by immersion of the curette into a conventional fixative appropriate for such study. Exact cross sections through the cilia afford the optimal view for evaluation. Particular care should be given to the evaluation of dynein arms inasmuch as a regular spacing of approximately 24 nm occurs between the adjacent dynein arms positioned along the length of individual microtubules. Thus, a cross section through an

individual cilium may pass through some arms and miss others suggesting that some arms are missing. To avoid this misinterpretation, an adequate number of cilia must be examined.

Primary Ciliary Dyskinesia In Pediatrics

The clinical manifestations of PCD are several and can generally be appreciated in view of the diminished ciliary function at the affected site. Babies with PCD may present with respiratory distress and mucopurulent nasal secretions and cough. *Hemophilus influenzae* is a commonly isolated pathogen although other pathogenic bacteria also appear.⁶³⁻⁶⁷ These early infections are a prelude to chronic or recurrent sinus and bronchial infections.^{67, 68} In childhood, nasal secretions and the development of nasal polyposis are continuing problems and may mandate tonsillectomy, adenoidectomy, and/or other surgical interventions.^{64, 69-71} Atelectasis and bronchiectasis also may begin early in childhood.^{64, 72} Among children with PCD, recurrent otitis media with effusion and occlusion of the eustachian tubes are common; however, these problems generally attenuate with age although some hearing loss may occur.^{64, 73} In a similar vein, patients with PCD have a poor sense of smell, a factor probably less related to the structural dysmorphology of the cilia than to nasal congestion and/or inadequate mucus flow.⁷⁴

Although the ependyma of the brain possesses a ciliated epithelium, overt neurologic manifestations of PCD relative to pediatric populations are not well known. A single case report documents PCD in a 12-year-old boy who had hydrocephalus as an infant.⁷⁵ The condition was treated by surgical intervention and the youth had normal intelligence. Severe headaches often are reported by patients with PCD,⁷⁶ a symptom that could be reflective of reduced circulation of cerebrospinal fluid associated with compromised ciliary activity. However, it is equally plausible that chronic sinus infections among PCD patients also may contribute to headache.⁷⁴

Clinical Aspects of Infertility In Primary Ciliary Dyskinesia

The upper respiratory tract and otologic manifestations of PCD are among the most significant in pediatric populations. However, problems of infertility, while not exclusive of pediatric patients, are a pertinent concern to adult patients. In general, males with PCD have spermatozoa with little or no motility and are thus thought to be generally infertile^{42, 76, 77}; however, there is anecdotal evidence that men with PCD have fathered children. Thus, the issue of male infertility in PCD remains somewhat unresolved.

The role of ciliary motility in the infertility of females with PCD also is suggestive but not definitive. Inasmuch as collateral mechanisms may contribute, ciliary competence appears to be not absolutely requisite for the transport of the ovum. One study reported that among one group of 12 women with PCD seeking to conceive over a period of 3 years, three fourths had not been able to do so.⁷⁷ In a separate report,⁷⁸ the

immotility of both respiratory and oviductal cilia as well as the absence of dynein arms was documented in an infertile woman with KS.

Animal Models of Primary Ciliary Dyskinesia

A number of experimental models of ciliary and flagellar structure and function have been identified. Among these, several ciliary mutants among certain eukaryotic protists, particularly *Chlamydomonas moewusii* and *Paramecium tetraurelia* are known.^{79, 80} Among mammals, mutant mice with varying phenotypic expressions of some of the features of PCD have been described.⁸¹ However, the most promising mammalian model of PCD identified to date appears to be among dogs in which classical clinical signs and ultrastructural findings characteristic of PCD have been documented.⁸²

The Relationship (or Lack Thereof) of Primary Ciliary Dyskinesia to Other Chronic and/or Congenital Diseases

The relationship of KS to PCD is well established. While it is clear that PCD can occur in the absence of situs inversus, the clinical findings of chronic sinusitis and bronchiectasis develop from early life and are characteristic. Also, because of the apparent autosomal recessive nature of inheritance,^{42, 46, 83} PCD with situs inversus may appear among other close relatives.

Individuals with situs inversus have a normal albeit reversed body asymmetry, whereas in polysplenia and asplenia syndromes there exists a mirror-image symmetry of the viscera. Several reports have identified familial relationships^{84, 85} in which one individual has polysplenia or asplenia, the other has KS, and both exhibit dysmorphic cilia with defective dynein arms. Thus, there appears to be an apparent, although presently unclarified, relationship between polysplenic and asplenic syndromes and PCD.

The hallmarks of cystic fibrosis (CF) are pancreatic insufficiency, altered sweat chloride, viscid mucus secretions, and severe chronic respiratory disease. It is the most frequent lethal heritable syndrome among Caucasians and its respiratory manifestations may mirror those of PCD to the point of occasional misdiagnosis. However, unlike PCD patients, mucociliary clearance and apparent effective ciliary activity can be demonstrated in individuals with CF.^{86, 87} Additionally, the ultrastructure of the cilia among patients with CF appears normal with the caveat that certain acquired ciliary defects most likely resulting from infection may appear. However, it is clear that an index ciliary lesion specific for CF does not occur, whereas individuals with PCD do express disease-specific ultrastructural level lesions. While there is no direct evidence of a genetic link or relationship between CF and PCD; it is noteworthy that two case reports have documented individuals with both CF and PCD.^{88, 89}

Young's syndrome⁹⁰ is a clinical entity thought possibly to be of genetic origin.⁹¹ It is mentioned because of its similarity to CF and to PCD in its pulmonary manifestations and its relevance to male infertility. However, patients with Young's syndrome appear to have generally normal

cilia.^{92, 93} Young's syndrome males exhibit normal spermiogenesis,^{91, 94} their infertility being derived from azoospermia due to an idiopathic obstruction of the vasa efferentia. While some of the clinical findings in Young's syndrome also are similar to those seen in CF, the characteristics of the secretions are normal and there is no pancreatic insufficiency.

Retinitis pigmentosa (RP), a hereditary disease characterized by retinal degeneration, may have as its pathophysiologic basis ciliary defects in the modified cilia of the retina. Several reports have documented the increased prevalence of abnormal cilia in the nasal epithelium as well as reduced fertility of individuals with RP.^{44, 95} There is a subgroup of individuals with RP who experience early deafness which similarly has been postulated to be due to a defect of the sensory hairs on the cells of the inner ear⁹⁵ and is called Usher's syndrome. The possibility of a relationship between RP and/or Usher's syndrome and PCD is intriguing. Ciliary pathophysiology appears as a plausible basis for all three conditions although RP and Usher's syndrome appear to affect mainly the monociliated sensory cells whereas PCD involves multiciliated cells. Both conditions exhibit a reduced level of fertility and similar ultrastructural level ciliary and/or flagellar lesions. There is a clear commonality between the two conditions and it appears likely they are related in some as yet undetermined way.

Acquired Ciliary Defects

Although the etiology of acquired ciliary defects can be distinguished from congenital forms, their clinical and ultrastructural manifestations often may appear disturbingly similar. Because congenital ciliary abnormalities are hereditary, important distinguishing factors are their quantitative abundance and anatomic pervasiveness whereas acquired ciliary defects generally are more structurally diverse and quantitatively variable. Acquired ciliary defects and concomitant mucociliary dysfunction appear in the respiratory airways as a consequence of various insults such as irritation by air pollutants and infection by microbial pathogens. This spectrum of acquired ciliary defects is different in acute and chronic disease states.

Acquired Ciliary Defects in Acute Respiratory Disease: Experimental and Clinical Observations

Acute injury to the airway mucosa of otherwise normal healthy humans by toxic or infectious agents is inevitable. The severity of the insult may range from mild discomfort to a fatal encounter. While the highly coordinated mucociliary escalator represents a first line of defense to the entire respiratory system, the ciliated cells composing much of the mucosal border are themselves vulnerable to injury. It appears likely that certain acquired ciliary defects develop immediately or within a relatively short time following an insult, whereas others appear to be derived along different developmental lines and may require a more extended period of time for their expression.

Although compound cilia are hallmark features of chronic respiratory diseases, they also have been documented as ciliary defects of acute cause in inhalation toxicology studies among human subjects.⁹⁶ While the mechanism(s) whereby compound cilia are formed is uncertain, it appears likely that the membrane integrity of adjacent extant or developing cilia may be compromised in a way that facilitates membrane fusion. Other exposure studies of ambient air pollutants⁹⁷⁻⁹⁹ also have suggested that compromised mucociliary clearance, possibly brought about by ciliary injury, plays a role in air pollutant-induced airway disease.

Pathogenic microbiologic agents also have been found to induce ciliary defects in airway epithelium. Carson et al.¹⁰⁰ demonstrated that experimental infection of tracheal organ cultures by *Mycoplasma pneumoniae* elicited changes in the configuration of the host tissue ciliary membrane. Collier and Baseman¹⁰¹ had previously shown the attenuation of ciliary beat in tracheal organ cultures similarly infected with this agent. The consistency of these findings with clinical studies on naturally acquired *M. pneumoniae* disease¹⁰² illustrates the efficacy and importance of experimental models in elucidating basic pathophysiologic mechanisms. The basis for mycoplasmal disruption of ciliary structure and function may reside in two characteristics: its production of hydrogen peroxide as a respiratory end product¹⁰³ and the ability of the organism to inhibit host cell catalase.¹⁰⁴ Similar mechanisms also may be operative in bacterial infections.

In addition to the potential for ciliary membrane damage, internal structures of the cilium may be disposed to structural modifications by acute insults to the airways as well. Carson et al.¹⁰⁵ reported the appearance of aberrations of microtubular configuration in the nasal mucosal cilia of a group of children enrolled in day care and presenting with naturally acquired, culture-documented viral infections of the upper respiratory tract (Fig 12). Because of the complexity of organization of the various axonemal elements, it was proposed that these aberrant cilia were derived from errors of assembly during ciliogenesis rather than a de novo change in the microtubular configuration of mature, extant cilia. The specific mechanism whereby virus infection might lead to such ciliary changes remains uncertain.

Acquired Ciliary Defects in Chronic Respiratory Disease

There is good evidence that recurrent infection or other chronic insults to the respiratory airways exacerbate existing chronic disease states and increase the risk for the development of chronic respiratory disease among healthy individuals. Features of PCD and the clinical and ultrastructural hallmarks facilitate diagnosis of this condition. However, individuals with PCD also frequently exhibit a spectrum of ciliary defects that can be distinguished as acquired. Additionally, individuals with other non-PCD chronic respiratory diseases including carcinoma, cystic fibrosis, bronchiectasis, and recurrent infection also may exhibit similar ciliary defects though acquired.

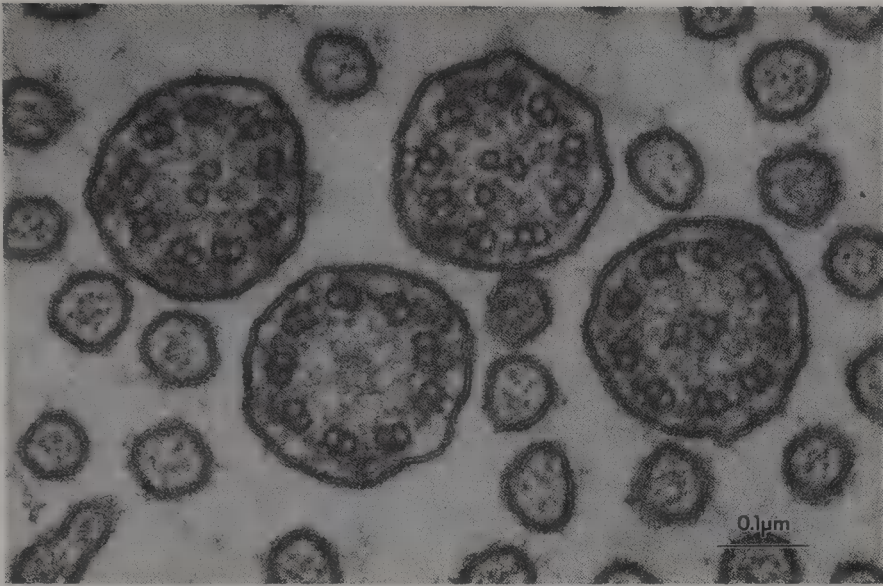


FIG 12.

Cross sections of cilia from a normal (viz. without chronic disease) child with an acute viral upper respiratory infection illustrating microtubular additions and deletions and the misalignment of central microtubular pairs of adjacent cilia.

Changes in ciliary membrane structure are among most common of the acquired ciliary defects seen in chronic respiratory disease. Compound cilia and protrusions of the ciliary membrane are a prominent feature of a variety of chronic respiratory diseases (Figs 13 and 14).¹⁰⁶⁻¹⁰⁹ Recurrent bacterial infections are a characteristic trait of many chronic airway diseases and some of these agents appear capable of producing metabolites that interfere with ciliary beat¹¹⁰⁻¹¹¹ and may disrupt ciliary structural integrity.¹¹² A further deleterious factor occurring in the presence of bacterial infection and in allergic responses is the interference of ciliary activity by leukocyte enzymes.^{113, 114} Thus, the individual with chronic disease is caught in an unfortunate cycle of harboring pathogens capable of altering ciliary structure and function as well as generating a defective host defense against those pathogens that also may be implicated in compromising ciliary function.

While changes in ciliary membrane structure appear to be among the most common acquired pathologic features of cilia in chronic disease including PCD, axonemal microtubular alterations also have been reported.¹¹⁵ The mechanisms leading to the appearance of these patterns in individuals with chronic respiratory disease may be similar to that proposed by Carson et al.¹⁰⁵ in acute viral upper respiratory infections where conditions or pathogens capable of effecting errors in ciliogenesis are present. For example, an experimental study by Heino and Laitenen¹¹⁶ documented the ciliogenesis-altering toxic effects of oxygen on rats exposed to



FIG 13.

Compound (CC) and internalized (IC) cilia in a child with bronchiectasis of unknown cause. The appearance of compound cilia is highly correlated with many chronic airways diseases. Internalized cilia are less prevalent and may represent anomalous primary cilia.

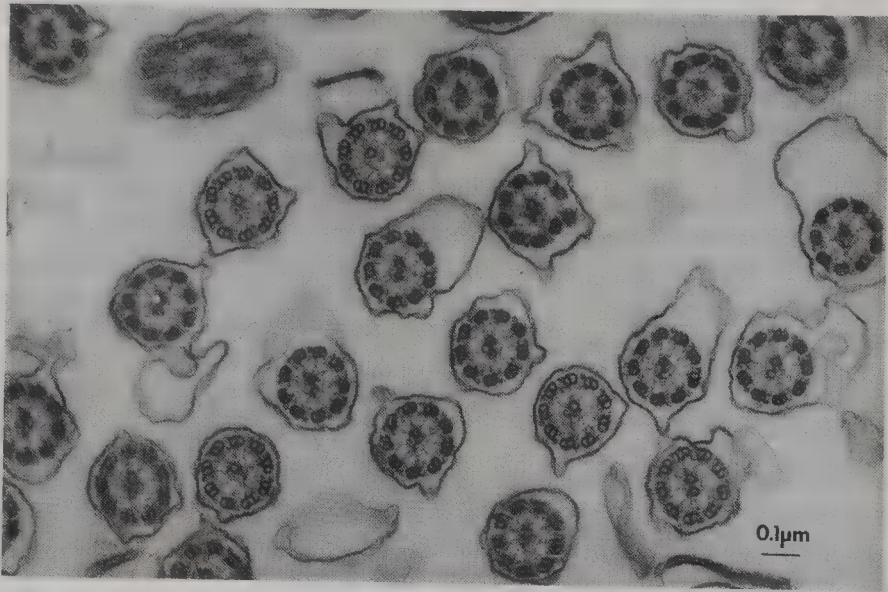


FIG 14.

Aberrations of ciliary membrane integrity are commonly seen in chronic airways diseases particularly among individuals with bronchiectasis. This photomicrograph illustrates protrusions of the ciliary membranes in the nasal epithelium of a child with PCD that are reflective of this acquired ciliary defect.

pure oxygen for up to 24 hours. A subsequent clinical study by Lee and colleagues¹¹⁷ demonstrated the appearance of ciliary microtubular defects consistent with errors of ciliogenesis in the nasal epithelium of a child with bronchopulmonary dysplasia who had been receiving supplemental oxygen for an extended period.

Several reports have documented other noteworthy changes in ciliary ultrastructure and alignment that appear to be acquired in various chronic disease states. These include the appearance of excess matrix surrounding the internal axonemal elements¹¹⁸ and the appearance of ciliary shafts sequestered in the cytoplasm (see Fig 13).¹¹⁹

While the changes previously described are well documented, clinicians should exercise caution in analyzing the appearance of such changes and in concluding their suspected relevance to clinical presentations. Several reports^{118, 120, 121} have pointed out that dysmorphic cilia can be found in specimens of ciliated mucosa from healthy asymptomatic subjects.

Summary

Considerable progress has been made in achieving a perspective of the pathophysiology of ciliary defects in human disease in the interval between Siewert's and Kartagener's early descriptions of KS. Not only have we achieved a better understanding of some of the mechanisms involved in the pathogenesis of congenital ciliary syndromes, but also we have come to appreciate a new spectrum of ciliary defects, those of acquired etiology. The advent of modern electron microscopy has been a significant element to this progress and its importance is reflected by the numerous studies of ciliary defects that have come in the wake of the pioneering ultrastructural investigations of Afzelius and colleagues. However, each discovery appears to generate additional questions. This is indicative of the importance of the subject to both basic and clinical science and of the vitality of the investigators in this field as a comprehensive understanding of ciliary diseases is sought.

Acknowledgments

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References

1. Friedmann I, Bird ES: Ciliary structure, ciliogenesis, microvilli: Electron microscopy of the mucosa of the upper respiratory tract. *Laryngoscope* 1971; 81:1852-1868.
2. Hilding DA, Heywood P: Ultrastructure of middle ear mucosa and organization of ciliary matrix. *Ann Otol* 1971; 80:306-312.
3. Harada Y: Scanning electron microscopic study on the distribution of epithelial cells in the eustachian tube. *Acta Otolaryngol* 1977; 83:284-290.
4. Morita I: Some observations on the fine structure of the human ductuli efferentes. *Arch Histol Japon* 1966; 26:341-365.
5. Ludwig H, Wolf H, Metzger H: Ultrastructure of the luminal surface of fallopian tube in the scanning electron microscope. *Arch Gynakol* 1972; 212:380-396.
6. More IAR, Masterton RG: The role of oestrogen in the control of ciliated cells of the human endometrium. *J Reprod Fertil* 1976; 47:19-24.
7. Worthington RC, Cathcart RS: Ependymal cilia: Distribution and activity in the adult human brain. *Science* 1963; 139:221-222.
8. Moran DT, Rowley III JC, Jafek BW, et al: The fine structure of the olfactory mucosa in man. *J Neurocytol* 1982; 11:721-746.
9. Flock A, Flock B, Murray E: Studies on the sensory hairs of receptor cells in the inner ear. *Acta Otolaryngol* 1977; 83:85-91.
10. Bloom W, Fawcett DW: *Textbook of Histology*. Philadelphia, WB Saunders Co, 1975.
11. Barnes BG: Ciliated secretory cells in the pars distalis of the mouse hypophysis. *J Ultrastr Res* 1961; 5:453-467.
12. Scherft JP, Daems WTh. Single cilia in chondrocytes. *J Ultrastruc Res* 1967; 19:546-555.
13. Wheatley DN: Cilia and centrioles of the rat adrenal cortex. *J Anat* 1967; 101:223-237.
14. Flood PR, Totland GK: Substructure of solitary cilia in mouse kidney. *Cell Tissue Res* 1977; 183:281-290.
15. Myklebust R, Engedal H, Saetersdal TS, et al: Primary 9+0 cilia in the embryonic and the adult human heart. *Anat Embryol* 1977; 151:127-139.
16. Stubblefield E, Brinkley BR: Cilia formation in Chinese hamster fibroblasts in vitro as a response to Colcemid treatment. *J Cell Biol* 1966; 30:645-652.
17. Sorokin SP: Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J Cell Sci* 1968; 3:207-230.
18. Kirkpatrick JB, Hyams L, Thomas VL, et al: Purification of intact microtubules from brain. *J Cell Biol* 1970; 47:384-394.
19. Shelanski ML, Taylor EW: Isolation of a protein sub-unit from microtubules. *J Cell Biol* 1967; 34:549-554.
20. Shelanski ML, Taylor EW: Properties of the protein subunit of central-pair and outer-doublet microtubules of sea urchin flagella. *J Cell Biol* 1968; 38:304-315.
21. Gibbons IR, Rowe AJ: Dynein: A protein with adenosine triphosphatase activity from cilia. *Science* 1965; 149:424-426.
22. Goodenough UW, Heuser JE: Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. *J Cell Biol* 1985; 100:2008-2018.

23. Goodenough UW, Heuser JE: Substructure of the outer dynein arm. *J Cell Biol* 1982; 95:798–815.
24. Gibbons IR: Chemical dissection of cilia. *Arch Biol (Liege)* 1965; 76:317–352.
25. Warner FD: The fine structure of the ciliary and flagellar axoneme, in Sleight MA (ed): *Cilia and Flagella*. New York, Academic Press, 1974, pp 11–38.
26. Gilula N, Satir P: The ciliary necklace: A ciliary membrane specialization. *J Cell Biol* 1972; 53:494–509.
27. Afzelius BA: Electron microscopy of the sperm tail. *J Biophys Biochem Cytol* 1959; 5:269–278.
28. Satir P: Studies on cilia: II. Examination of the distal region of the ciliary shaft and the role of the filaments in motility. *J Cell Biol* 1965; 26:805–834.
29. Dirksen ER, Zeira M: Microtubule sliding in cilia of the rabbit trachea and oviduct. *Cell Motil* 1981; 1:247–260.
30. Satir P, Wais-Steider J, Lebduska S, et al: The mechanochemical cycle of the dynein arm. *Cell Motil* 1981; 1:303–327.
31. Rosenbaum J, Child F: Flagellar regeneration in protozoan flagellates. *J Cell Biol* 1967; 34:345–364.
32. Rosenbaum JL, Moulder JE, Ringo DL: Flagellar elongation and shortening in *Chlamydomonas*: I. The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J Cell Biol* 1969; 41:600–619.
33. Witman GB: The site of in vivo assembly of flagellar microtubules. *Ann NY Acad Sci* 1975; 253:178–191.
34. Dentler W, Rosenbaum JL: Flagellar elongation and shortening in *Chlamydomonas*: III. Structures attached to the tips of flagellar microtubules and their relationship to the directionality of flagellar microtubule assembly. *J Cell Biol* 1977; 74:747–759.
35. Lefebvre PA, Silflow CD, Wieben ED, et al: Increased levels of mRNAs for tubulin and other flagellar proteins after amputation or shortening of *Chlamydomonas* flagella. *Cell* 1980; 20:469–477.
36. McKeithan TW, Lefebvre PA, Silflow CD, et al: Multiple forms of tubulin in *Polytomella* and *Chlamydomonas*: Evidence for a precursor of flagellar α -tubulin. *J Cell Biol* 1983; 96:1056–1063.
37. Green LL, Dove WF: Tubulin proteins and RNA during the myxamoeba-flagellate transformation of *Physarum polycephalum*. *Mol Cell Biol* 1984; 4:1706–1711.
38. Piperno G, Fuller M: Monoclonal antibodies specific for an acetylated form of α -tubulin recognize the antigen in cilia and flagella from a variety of organisms. *J Cell Biol* 1975; 101:2085–2094.
39. Curtis LN, Carson JL, Collier AM, et al: Features of developing ferret tracheal epithelium: Ultrastructural observations of in vivo and in vitro differentiation of ciliated cells. *Exp Lung Res* 1987; 13:223–240.
40. Keenan KP, Wilson TS, McDowell EM: Regeneration of hamster tracheal epithelium after mechanical injury: IV. Histochemical, immunocytochemical, and ultrastructural studies. *Virchows Arch (Cell Pathol)* 1983; 43:213–240.
41. Carson JL, Collier AM, Knowles MR, et al: Morphometric aspects of ciliary distribution and ciliogenesis in human nasal epithelium. *Proc Nat Acad Sci USA* 1981; 69:696–6999.
42. Afzelius BA, Eliasson R, Johnsen O, et al: Lack of dynein arms in immotile human spermatozoa. *J Cell Biol* 1975; 66:225–232.

43. Afzelius BA: A human syndrome caused by immotile cilia. *Science* 1976; 193:317-319.
44. Arden GB, Fox B: Increased incidence of abnormal nasal cilia in patients with retinitis pigmentosa. *Nature* 1979; 279:534-536.
45. Wakefield StJ, Waite D: Abnormal cilia in Polynesians with bronchiectasis. *Am Rev Respir Dis* 1980; 121:1003-1010.
46. Sturgess JM, Thompson MW, Czegledy-Nagy E, et al: Genetic aspects of immotile cilia syndrome. *Am J Med Genet* 1986; 25:149-160.
47. Siewert A: Über einen Fall von Bronchiektasien bei einem Patienten mit situs inversus viscerum. *Klin Wochenschr* 1904; 41:139-141.
48. Kartagener M: Pathogenese der bronchiektasien bei situs viscerus inversus. *Beitr Klin Tuberk* 1933; 83:489-501.
49. Eliasson R, Mossberg B, Camner P, et al: The immotile-cilia syndrome: A congenital ciliary abnormality as an etiologic factor in chronic airway infections and male sterility. *N Engl J Med* 1977; 297:1-6.
50. Rossman CM, Forrest JB, Lee RMKW, et al: The dyskinetic cilia syndrome: Ciliary motility in immotile cilia syndrome. *Chest* 1980; 78(suppl):580-582.
51. Pedersen M, Mygind N: Ciliary motility in the "immotile cilia syndrome." *Br J Dis Chest* 1980; 74:239-244.
52. Sleight MA: Primary ciliary dyskinesia. *Lancet* 1981; 2:476.
53. Camner P, Mossberg B, Afzelius BA: Evidence for congenitally non-functioning cilia in the tracheobronchial tract in two subjects. *Am Rev Respir Dis* 1975; 112:807-809.
54. Schneeberger EE, McCormack J, Issenberg HJ, et al: Heterogeneity of ciliary morphology in the immotile-cilia syndrome in man. *J Ultrastr Res* 1980; 73:34-43.
55. Neustein HB, Nickerson B, O'Neal M: Kartagener's syndrome with absence of inner dynein arms of respiratory cilia. *Am Rev Respir Dis* 1980; 122:979-981.
56. Nielsen MH, Pedersen M, Christensen B, et al: Blind quantitative electron microscopy of cilia from patients with primary ciliary dyskinesia and from normal subjects. *Eur J Resp Dis* 1983; 64(suppl 127):19-30.
57. Rooklin AR, McGeady SJ, Mikaelian DO, et al: The immotile cilia syndrome: A cause of recurrent pulmonary disease in children. *Pediatrics* 1980; 66:526-531.
58. Herzon FS, Murphy S: Normal ciliary ultrastructure in children with Kartagener's syndrome. *Ann Otol* 1980; 89:81-83.
59. Sturgess JM, Chao J, Wong J, et al: Cilia with defective radial spokes: A cause of human respiratory disease. *N Engl J Med* 1979; 300:53-56.
60. Sturgess JM, Chao J, Turner JAP: Transposition of ciliary microtubules. *N Engl J Med* 1980; 303:318-322.
61. Rutland J, Dewar A, Cox T, et al: Nasal brushing for the study of ciliary ultrastructure. *J Clin Pathol* 1982; 35:357-359.
62. Alford BR, Douglas RG Jr, Couch RB: Atraumatic biopsy of nasal mucosa. *Arch Otolaryngol* 1969; 90:88-92.
63. Corkey CWB, Levison H, Turner JAP: The immotile cilia syndrome: A longitudinal survey. *Am Rev Respir Dis* 1981; 124:544-548.
64. Ernstson S, Afzelius BA, Mossberg B: Otologic manifestations of the immotile-cilia syndrome. *Acta Otolaryngol* (Stockholm) 1984; 97:83-92.
65. Finnstrom O, Odkvist L, Afzelius BA: The immotile cilia syndrome in a newborn infant. *Int J Pediatr Otolrhinol* 1980; 2:33-37.

66. Whitelaw A, Evans A, Corrin B: Immotile cilia syndrome: A new cause of neonatal respiratory distress. *Arch Dis Child* 1981; 56:432-435.
67. Van der Baan S, Veerman AJP, Weltevreden EF, et al: Kartagener's syndrome: Clinical symptoms and laboratory studies. *Eur J Respir Dis* 1983; 64(suppl 127):91-95.
68. Mossberg B, Afzelius BA, Eliasson R, et al: On the pathogenesis of obstructive lung disease. *Scand J Resp Dis* 1978; 59:55-65.
69. Turner JAP, Corkey CWB, Lee YC, et al: Clinical expression of immotile cilia syndrome. *Pediatrics* 1981; 67:805-810.
70. Mygind N, Pedersen M: Nose-, sinus-, and ear-symptoms in 27 patients with primary ciliary dyskinesia. *Eur J Resp Dis* 1983; 64(suppl 127):96-101.
71. Mygind N, Pedersen M, Nielsen MH: Primary and secondary ciliary dyskinesia. *Acta Otolaryngol* 1983; 95:688-694.
72. Pullan CR, Robertson DM, Robinson AD, et al: Investigation of children with abnormal cilia. *Eur J Respir Dis* 1983; 64(suppl 128):466-469.
73. Mygind NG, Pedersen M, Toremalm NG: Lazy cilia make the otologist busy. *Clin Otolaryngol* 1983; 8:148-150.
74. Afzelius BA: The immotile-cilia syndrome: A microtubule-associated defect. *CRC Crit Rev Biochem* 1985; 19:63-87.
75. Greenstone MA, Jones RWA, Dewar A, et al: Hydrocephalus and primary ciliary dyskinesia. *Arch Dis Child* 1984; 59:481-482.
76. Afzelius BA: The immotile-cilia syndrome and other ciliary diseases. *Int Rev Exp Pathol* 1979; 19:1-43.
77. Afzelius BA, Eliasson R: Male and female infertility problems in the immotile-cilia syndrome. *Eur J Resp Dis* 1983; 64(suppl 127):144-147.
78. McComb P, Langley L, Villalon M, et al: The oviductal cilia and Kartagener's syndrome. *Fertil Steril* 1986; 46:412-416.
79. Byrne BJ, Byrne BC: An ultrastructural correlate of the membrane mutant "paranoiac" in *Paramecium*. *Science* 1978; 199:1091-1093.
80. Lewin RA: Mutants of *Chlamydomonas moewusii* with impaired motility. *J Gen Microbiol* 1954; 11:358-363.
81. Bryan JHD: The immotile cilia syndrome: Mice versus man. *Virchows Arch Pathol Anat* 1983; 399:265-275.
82. Wilsman NJ, Morrison WB, Farnum CE, et al: Microtubular protofilaments and subunits of the outer dynein arm in cilia from dogs with primary ciliary dyskinesia. *Am Rev Respir Dis* 1987; 135:137-143.
83. Afzelius BA: Genetical and ultrastructural aspects of the immotile-cilia syndrome. *Am J Hum Genet* 1981; 33:852-864.
84. Schidlov DV, Katz SM, Turtz MG, et al: Polysplenia and Kartagener syndromes in a sibship. *J Pediatr* 1982; 100:401-403.
85. Niikawa N, Kohsaka S, Mizumoto M, et al: Familial clustering of situs inversus totalis and asplenia and polysplenia syndromes. *Am J Med Genet* 1983; 16:43-47.
86. Kollberg H, Mossberg B, Afzelius BA, et al: Cystic fibrosis compared with the immotile-cilia syndrome. *Scand J Resp Dis* 1978; 59:297-306.
87. Rutland J, Cole PJ: Nasal mucociliary clearance and ciliary beat frequency in cystic fibrosis compared with sinusitis and bronchiectasis. *Thorax* 1981; 36:654-658.
88. Brown NM, Smith AN: Kartagener's syndrome with fibrocystic disease. *Br Med J* 1959; 5154:725-728.

89. Burnell RH, Robertson EF: Cystic fibrosis in a patient with Kartagener's syndrome. *Am J Dis Child* 1974; 127:746-747.
90. Young D: Surgical treatment of male infertility. *J Reprod Fert* 1970; 23:541-542.
91. Handelsman DJ, Conway AJ, Boyland LM, et al: Young's syndrome: Obstructive azoospermia and chronic sinopulmonary infections. *N Engl J Med* 1984; 310:3-9.
92. Hendry WF, Knight RW, Whitfield HN, et al: Obstructive azoospermia: Respiratory function tests, electron microscopy, and the results of surgery. *Br J Urol* 1978; 50:598-604.
93. Hendry WF, Whitfield HN, Stansfeld AG, et al: Defects in Young's syndrome and Kartagener's syndrome. *Lancet* 1978; 2:1152.
94. Neville E, Brewis R, Yeates WK, et al: Respiratory tract disease and obstructive azoospermia. *Thorax* 1983; 38:929-933.
95. Hallgren B: Retinitis pigmentosa combined with congenital deafness. *Acta Psychiatr Neurol Scand* 1959; 34(suppl 138):1-101.
96. Carson JL, Collier AM, Hu S, et al: The appearance of compound cilia in the nasal mucosa of normal human subjects following acute, in vivo exposure to sulfur dioxide. *Environ Res* 1987; 42:155-165.
97. Asmundsson T, Kilburn KH, McKenzie WN: Injury and metaplasia of airway cells due to SO₂. *Lab Invest* 1973; 29:41-53.
98. Heller RF, Gordon RE: Chronic effects of nitrogen dioxide on cilia in hamster bronchioles. *Exp Lung Res* 1986; 10:137-152.
99. Boatman ES, Sato S, Frank R: Acute effects of ozone on cat lungs: II. Structural. *Am Rev Respir Dis* 1974; 110:157-169.
100. Carson JL, Collier AM, Clyde WA Jr: Ciliary membrane alterations occurring in experimental *Mycoplasma pneumoniae* infection. *Science* 1979; 206:349-351.
101. Collier AM, Baseman JB: Organ culture techniques with mycoplasmas. *Ann NY Acad Sci* 1973; 225:277-289.
102. Collier AM, Clyde WA Jr: Appearance of *Mycoplasma pneumoniae* in lungs of experimentally infected hamsters and sputum from patients with natural disease. *Am Rev Respir Dis* 1974; 110:765-773.
103. Cohen G, Somerson NL: *Mycoplasma pneumoniae*: Hydrogen peroxide secretion and its possible role in virulence. *Ann NY Acad Sci* 1967; 143:85-87.
104. Almagor M, Yatziv S, Kahane I: Inhibition of host cell catalase by *Mycoplasma pneumoniae*: A possible mechanism for cell injury. *Infect Immun* 1983; 41:251-256.
105. Carson JL, Collier AM, Hu SS: Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. *N Engl J Med* 1985; 312:463-468.
106. Corbeel L, Cornillie F, Lauweryns J, et al: Ultrastructural abnormalities of bronchial cilia in children with recurrent airway infections and bronchiectasis. *Arch Dis Child* 1981; 56:929-933.
107. Cutz E, Levison H, Cooper DM: Ultrastructure of airways in children with asthma. *Histopathology* 1978; 2:407-421.
108. Katz SM, Holsclaw DS Jr: Ultrastructural features of respiratory cilia in cystic fibrosis. *Am J Clin Pathol* 1980; 73:682-685.
109. Ailsby RL, Ghadially FN: Atypical cilia in human bronchial mucosa. *J Pathol* 1975; 119:75-78.

110. Denny F: Effect of a toxin produced by *Haemophilus influenzae* on ciliated respiratory epithelium. *J Infect Dis* 1974; 129:93–100.
111. Wilson R, Roberts D, Cole P: Effect of bacterial products on human ciliary function in vitro. *Thorax* 1985; 40:125–131.
112. Hingley ST, Hastie AT, Kueppers F, et al: Disruption of respiratory cilia by proteases including those of *Pseudomonas aeruginosa*. *Infect Immun* 1986; 54:379–385.
113. Hastie AT, Loegering DA, Gleich GJ, et al: The effect of purified human eosinophil major basic protein on mammalian ciliary activity. *Am Rev Respir Dis* 1987; 135:848–853.
114. Tegner H, Ohlsson K, Toremalm NG, et al: Effect of human leukocyte enzymes on tracheal mucosa and its mucociliary activity. *Rhinology* 1979; 17:199–206.
115. Howell JT, Schochet SS, Goldman AS: Ultrastructural defects of respiratory tract cilia associated with chronic infections. *Arch Pathol Lab Med* 1980; 104:52–55.
116. Heino M, Laitinen LA: Oxygen exposure and extrapulmonary respiratory tract ciliogenesis in adult male rats. *Aviat Space Environ Med* 1982; 53:580–582.
117. Lee RMKW, Rossman CM, O'Brodovich H, et al: Ciliary defects associated with the development of bronchopulmonary dysplasia. *Am Rev Respir Dis* 1984; 129:190–193.
118. McDowell EM, Barrett LA, Harris CC, et al: Abnormal cilia in human bronchial epithelium. *Arch Pathol Lab Med* 1976; 100:429–436.
119. Torikata C, Takeuchi H, Yamaguchi H, et al: Abnormal cilia in the bronchial mucosa. *Virchows Arch A Pathol Anat Histol* 1976; 371:121–129.
120. Wisseman CL, Simel DL, Spock A, et al: The prevalence of abnormal cilia in normal pediatric lungs. *Arch Pathol Lab Med* 1981; 105:552–555.
121. Rossman CM, Lee RMKW, Forrest JB, et al: Nasal ciliary ultrastructure and function in patients with primary ciliary dyskinesia compared with that in normal subjects and in subjects with various respiratory diseases. *Am Rev Respir Dis* 1984; 129:161–167.

The Predictability of Insulin-Dependent Diabetes Mellitus

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Natural History

Insulin-dependent diabetes mellitus (IDDM) is one of the most common, debilitating chronic illnesses of children and young adults. Lifelong daily commitment to multiple insulin injections, blood glucose determinations, and dietary and exercise constraints is demanded in the hope of preventing the morbidity and the mortality associated with this disease. Unfortunately, retinopathy, nephropathy, neuropathy, myocardial infarction, stroke, and peripheral vascular disease are common sequelae. These complications result in a 50% mortality after 30 years of the disease.¹ Normalization of the metabolic abnormalities theoretically should prevent the development of these complications. Yet, despite recent advances in home glucose monitoring and insulin delivery methods, normoglycemia can rarely be sustained for more than a few months. Because conventional methods have failed to prevent the complications of IDDM, interest in possible means of disease prevention is intense. A major focus of research has been directed at the natural history and underlying autoimmune mechanisms leading to the loss of β -cell function.

Recent studies indicate that IDDM is not a disease of acute onset, as previously thought, but a disease with a prolonged preclinical phase.²⁻⁴ An autoimmune pathogenesis rather than an infectious etiology is now thought to be the mediator of islet cell destruction. The concept of autoimmunity in IDDM is based on the following:

1. Association with the immune response genes of the HLA-D region.⁵⁻¹⁷
2. Presence of autoantibodies to islet antigens, i.e., cytoplasmic (ICA)¹⁸⁻²⁰ or surface (ICSA),²¹⁻²² as well as antibodies to insulin (INSAB).²³⁻²⁶ In addition, autoantibodies to other endocrine tissues, e.g., thyroid microsomal antibodies (TMA),²⁷⁻³⁰ thyroglobulin antibodies, gastric parietal cell antibodies (PCA),³¹ and adrenocortical antibodies (ACA),³²⁻³³ are more frequent than expected among patients with IDDM.
3. Increased incidence of other autoimmune diseases in patients with IDDM or in their family members.³⁴⁻³⁶
4. Lymphocytic infiltration (insulitis) of the islets at the time of diagnosis.³⁷⁻³⁹
5. Abnormalities in cell-mediated immunity.⁴⁰⁻⁴⁵

Further evidence for immunologically mediated β -cell destruction comes from the recent reports of improved insulin production in association with immunosuppressive therapy in newly diagnosed IDDM.⁴⁶⁻⁵⁰ The availability of two animal models of IDDM, the nonobese diabetic mouse (NOD) and the bio-breeding rat (BB/W), has allowed further studies of the autoimmune process involved.⁵¹⁻⁵² Both animal models have diabetes resulting from severe insulin deficiency. The pancreatic insulitis lesion is similar to IDDM in man. Diabetes occurs spontaneously after a few months of life and is linked to the genes of the major histocompatibility complex.

A possible natural history of autoimmune IDDM is summarized in Figure 1. There is a genetic predisposition to the development of IDDM, although not everyone with these "at-risk" genes will develop IDDM, indicating an environmental factor(s) may be responsible for the initiation of the autoimmune process. Such an environmental insult would have to have occurred months or years before the onset of clinical symptoms, as a pre-clinical or asymptomatic phase has been identified.⁵³⁻⁶⁰ This phase is characterized by the presence of detectable immunologic abnormalities such as ICA and decreasing insulin secretion to various secretagogues, particularly to IV glucose. At the onset of clinical diabetes, as many as 90% of the insulin-producing β cells have been destroyed. This chapter summarizes the recent developments that have led to the conceptualization of IDDM as an autoimmune disease with a long prodromal phase.

Genetic Predisposition

Family History

Familial occurrences of IDDM are frequently encountered. The reported prevalence in siblings of children with IDDM is approximately 5%, a 15- to 20-fold greater risk than the general population.² The risk for the second

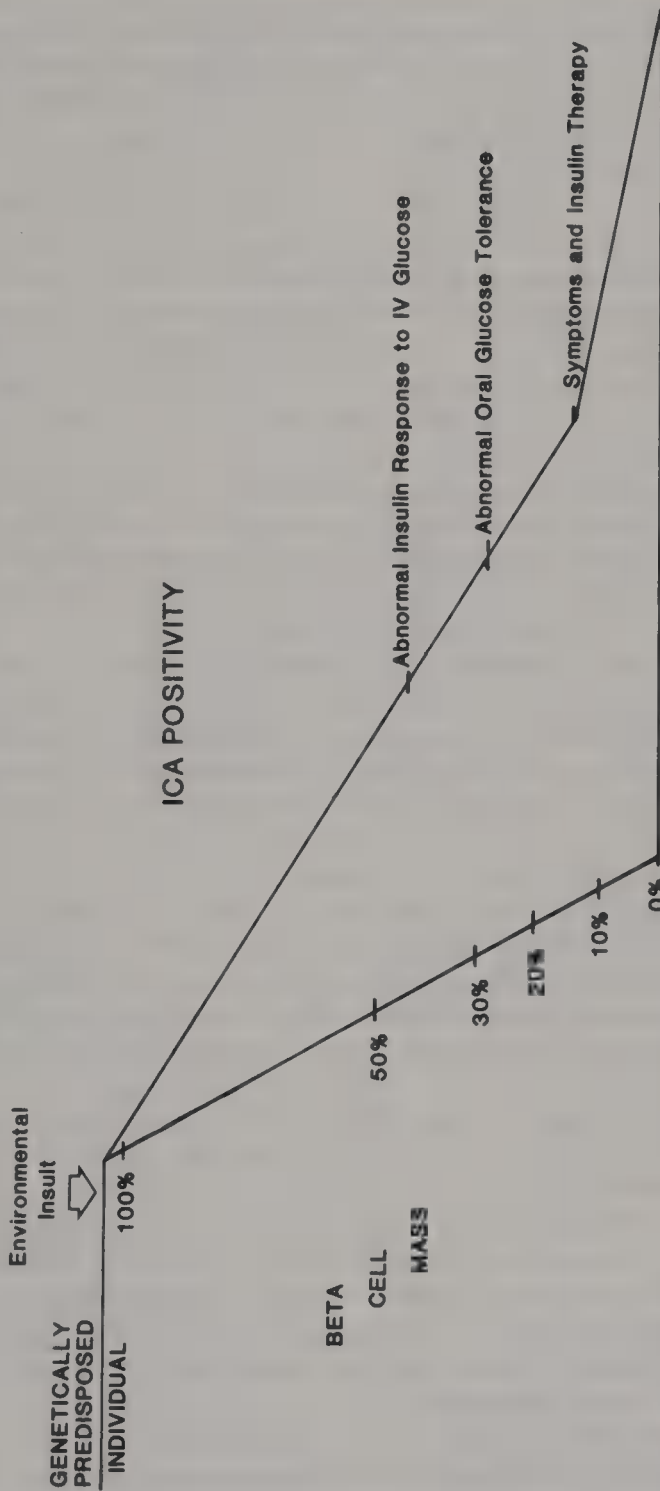


FIG 1.

The natural history of IDDM. In a genetically predisposed individual, an environmental insult initiates a process of autoimmune destruction of the pancreatic β cells that ultimately terminates in IDDM. The rate of progression is quite variable and is depicted by two different slopes. The metabolic indices are proposed to be dependent on the residual β cell mass.

of an identical twin pair to develop IDDM after diagnosis in the first is between 30% and 50%.^{2-4, 61} The prevalence of IDDM in offsprings of an IDDM parent varies from 2% to 6%. The higher frequency occurs if the father has IDDM.⁶² The majority of patients with IDDM, however, lack any history of the disease in their families.

HLA Genes

The association of IDDM with the B8 and B15 alleles of the B locus of the major histocompatibility region on the short arm of the sixth chromosome, was reported 15 years ago⁵ and confirmed.^{6, 7} More recently, the primary association has been shown to be in the D (DR) region. The B8 allele was found to be in linkage disequilibrium with the DR3 allele and the B15 allele with the DR4 allele.⁸ Nearly 95% of patients with IDDM have a DR3 or DR4 allele, with 40% heterozygous for both DR3 and DR4. On the other hand, 60% of the general population have either a DR3 or DR4 allele. Thus, one of the unanswered questions in the genetics of IDDM is why everyone with a DR3 or DR4 allele does not develop IDDM. It is rare that individuals with IDDM don't have a DR3 and/or DR4 allele, which are much less common in the nondiabetic. In individuals with only one DR3 or DR4, the DR1 allele is significantly more common than expected.¹⁰ Thus, DR1 appears to be an additional "at-risk" allele with the established diabetes-associated DR3 and DR4 alleles.¹⁰ Two alleles, DR2 and DR5, are rarely found in patients with IDDM and have been proposed as protective alleles.¹⁰

With the recent development of DNA probes used in restriction fragment length polymorphism (RFLP) analyses, groups have attempted to determine differences in the serologically defined DR3 or DR4 alleles between patients with or without IDDM. In addition, genes in the DQ region, a region tightly linked to the DR region, have been proposed as the "IDDM risk" genes.¹¹ Analyses of RFLP results to date have lead to several conclusions.

First, serologically defined DR and DQ haplotypes are heterogenous with respect to RFLPs. Many of the RFLPs were allelic, either alone or in combination with other fragments. Nepom and colleagues¹² separated the DR4-Dw4 DQw3 haplotype into 3.1 and 3.2 alleles on the basis of the presence (DQw3.1) or absence (DQw3.2) of a 3.7kb DQ β BamHI fragment. The DQw3.1 allele corresponded to the antigenic epitope TA10, which is specifically recognized by a monoclonal antibody. But Kim et al.¹³ concluded that this was a haplotype marker rather than a disease marker. Cohen-Haguehauer et al.¹⁴ identified a DR β TaqI 12.7kb fragment as part of an extended serologically defined B8-DR3 haplotype.

Second, the presence or absence of certain fragments or group of fragments have either a positive or negative association with IDDM. Owerbach et al.¹⁵ demonstrated that a DQ β BamHI 3.7kb fragment, alone or in com-

bination with other fragments, is significantly decreased in IDDM patients with a serologically defined DR4 allele. In a similar manner, we¹⁶ demonstrated, in homozygous DR4 or heterozygous DR3/DR4 individuals, that a specific pattern of DQ β fragments had a significant negative correlation with IDDM. Although DR2 is thought protective for IDDM, Cohen et al.¹⁷ demonstrated that lack of a DQ β EcoRI 2.2kb fragment correlated with IDDM among those patients with a DR2 allele.

HLA-DR-associated genes account for most of the genetic influence on IDDM susceptibility. However, other genetic factors associated with increased risk of developing IDDM include male gender, thyrogastric autoimmunity, immunoglobulin allotypes, and insulin gene polymorphisms.

Gender

In most autoimmune diseases, females tend to be affected at least twice as frequently as males.³⁵ However, in those who develop IDDM before the age of 5, males predominate.^{63, 64} A classification of IDDM into types 1a and 1b has been based on differences in sex and expression of autoimmune diseases.⁶⁵ Type 1a is characterized by early onset of IDDM, male sex, and decreased frequency of autoantibodies and associated autoimmune diseases. Type 1b is characterized by older age of onset of IDDM, female sex, and frequent association of autoantibodies and autoimmune diseases. The authors of this classification also proposed that type 1a is viral mediated whereas type 1b is the autoimmune form of IDDM. Whether this classification will survive after the ongoing longitudinal studies are evaluated remains to be seen. We doubt that IDDM can be classified so simply.

Warram et al.⁶² demonstrated a sexual bias in the transmission of IDDM from parent to offspring. Diabetes was five times more frequent in children of IDDM fathers than of affected mothers. In addition, parental gender affects the transmission of the HLA-DR "at-risk" alleles.⁶⁶ Transmission to offspring of paternal DR4 alleles was increased compared with maternal DR4 alleles, whereas transmission of the DR3 allele appeared to be equal from mothers and fathers.⁶⁶

Thyrogastric Autoimmune Genes

Patients with IDDM are at risk for other autoimmune diseases such as Hashimoto's thyroiditis, Graves' disease, and pernicious anemia.²⁷⁻³⁶ Family members of an IDDM proband also have an increased incidence of these autoimmune diseases.^{35, 36} Since IDDM segregates with certain HLA-DR genes, we were surprised to find that thyroiditis (and/or thyroid antibodies) and pernicious anemia (and/or atrophic gastritis and/or parietal cell antibodies) occur in siblings without any associations with IDDM or HLA genes.⁶⁷ Thyrogastric autoimmunities did not segregate with HLA

haplotypes as did IDDM. Thus, thyrogastric autoimmunity appears to be an additional risk factor for IDDM with a separate genetic system.^{67, 68}

Insulin Gene Polymorphisms

Initial studies of the human insulin gene on chromosome 11 suggested an association between IDDM and RFLPs in the region 5' to the gene.⁶⁹ This hypervariable region was classified into three groups by length of the DNA inserts. Class 1, 2 and 3 alleles contained approximately 45, 90, and 170 tandem base repeats, respectively. There was an increased frequency of the class 1 allele in IDDM patients, but no segregation with HLA-DR genes. Recent studies have failed to confirm this association.⁷⁰

Immunoglobulin Allotypes

Certain combinations of HLA-DR phenotype and immunoglobulin heavy-chain, constant region allotype increased the relative risk of developing Graves' disease or chronic active hepatitis.^{71, 72} Thus, certain immunoglobulin allotypes were increased in frequency in IDDM patients.^{73, 74} Immunoglobulin allotypes did not significantly influence the relative risk for IDDM associated with HLA-DR genes and probably contribute little to inherited susceptibility for the disease.

Environmental Factors

The importance of an environmental factor in the development of IDDM has been suggested from studies in monozygotic twins.⁶¹ Despite having the same genes as the affected twin, the second twin will develop IDDM only 30% to 50% of the time,⁶¹ suggesting that a chance environmental encounter may be involved. In the BB/W rat or NOD mouse animal models, the frequency of diabetes is never 100% even for animals reared on the same food and in identical environments.⁷⁵⁻⁷⁷

Viruses

With the recent demonstration of a prolonged preclinical phase, the role of viruses in the pathogenesis of IDDM needs to be reevaluated. The possibility that an infectious agent was causing IDDM was derived from animal models and the observation of a seasonal variation in the onset of IDDM.⁷⁸⁻⁸¹ Several viral agents (i.e., encephalomyocarditis virus and coxsackievirus B) are able to produce diabetes in mice.^{78, 79} One of the interesting phenomenon peculiar to these viruses is the need to passage them through pancreatic tissue in order to maintain their diabetogenicity.⁸² Serial passages through nonpancreatic tissue resulted in loss of diabetogenic effects.⁸² In humans, antibodies to coxsackievirus were more common in newly diagnosed patients with IDDM than in the general popula-

tion.⁸³ Our laboratory and others could not confirm this finding.^{84, 85} In several case reports, coxsackievirus was cultured from the stools and even the pancreas itself in newly diagnosed patients with IDDM.⁸⁶⁻⁸⁸ Mumps virus has also been implicated in the etiology of IDDM.⁸⁹ One report that ICA were frequently found in children after mumps infection has not been confirmed.⁹⁰ Obviously, there may be some cases of acute virally induced IDDM, but the role of viruses may be to acutely injure enough β cells to move one from marginal insulin reserve to inadequate.

On the other hand, the role of rubella in the etiology of IDDM appears to be different and might be a prototype of viral induction of the autoimmune process. Individuals who have been exposed to intrauterine rubella develop IDDM at a higher frequency than expected⁹¹; however, this occurs in the second and third decades, 10 to 20 years from the initial exposure to the rubella virus. Since rubella has profound effects on the immune system,⁹² perhaps the rubella virus initiates the autoimmune process that ultimately results in the destruction of the islets.

Ingestions: Toxins or Dietary

Animal models for chemically induced diabetes have been studied. The administration of streptozotocin (SZ) has been proposed as a model of chemically induced autoimmune diabetes because it results in lymphocytic infiltration of the islets and delayed β -cell necrosis.⁹³ Multiple small doses of SZ after an interval of 5 to 7 days result in insulinitis and hyperglycemia.⁹³ Single low doses of SZ can induce diabetes after variable lag periods but is dependent on the strain, age, and sex of the mice.⁹³ Whether the destructive processes are primarily immunologically mediated is questioned because studies have shown that similar doses of SZ produced diabetes in immunologically incompetent nude mice (unpublished observations). After a single low dose of SZ, the islets were shown to have been damaged before any lymphocytic infiltration occurred.⁹⁵ In humans, the ingestion of a rodenticide (Vacor) has produced an acute onset of diabetes in which ICSA were detected.⁹⁶ The immunologic component of this syndrome is still in doubt, as no other immunologic abnormalities were detected and the observation remains unconfirmed.

The original report of a protective effect of breast feeding on the development of IDDM has not been confirmed.⁹⁷ Other studies have addressed the possibility of a dietary factor in diabetes.⁹⁸⁻¹⁰⁰ Several studies in BB/W rats have demonstrated that substitution of dietary animal protein with vegetable protein prevented spontaneous diabetes.⁹⁸⁻¹⁰⁰ Investigators from Iceland proposed that the maternal ingestion of smoked mutton, a traditional Christmas food, might be a causative factor, as an excessive number of children born 8 to 9 months after Christmas developed IDDM.¹⁰¹ These provocative studies should be considered preliminary but underscore our need to search for a factor or factors that might initiate the immune response.

Immunologic Abnormalities to Predict IDDM

Immunologic abnormalities reported in IDDM can be grouped into humoral (antibody) and cellular (cell mediated), which occur long before the clinical onset of disease and support the belief that IDDM is an autoimmune disease.

Humoral Immunity

Islet Cell Cytoplasmic Antibodies

Several antibodies to islet cell antigens have been reported in patients with IDDM.¹⁸⁻²⁶ Antibodies to cytoplasmic antigens (ICA) are detected by indirect immunofluorescence using unfixed "snap frozen" human pancreatic tissue.¹⁸⁻²⁰ Differences in assays in various laboratories¹⁰²⁻¹⁰⁵ give somewhat different results and have been the impetus for three international workshops to standardize the method.^{106, 107} A similar process of standardization and quality control of the assay for antinuclear antibodies was done in the early stages of its development and allowed investigators to establish more quickly clinical significance and usefulness. Studies in which fixed tissue was used do not correlate with those using unfixed tissue and appear to detect ICA not relevant to IDDM.¹⁰⁵

ICA are present in approximately 75% of newly diagnosed patients with IDDM.²⁰ ICA are polyclonal, have both κ and λ chains, and are represented in all IgG subclasses with a predominance of IgG1.¹⁰⁸ In patients with IDDM, ICA decrease in frequency with increasing duration of IDDM. They are found less frequently in black patients with IDDM and are more common in patients who are DR3/DR4 heterozygotes.²⁰ The role of ICA in the pathogenesis of IDDM has yet to be defined; however, their importance is that the presence of ICA may precede the development of IDDM by more than 8 years.⁵³⁻⁶⁰

In a retrospective study of monozygotic twins who were initially discordant for IDDM, four of the five twins had ICA 5 to 8 years prior to their clinical onset of IDDM.⁵⁹ A number of groups have ongoing prospective studies of families with IDDM.⁵³⁻⁶⁰ The frequency of ICA in the nondiabetic family members of IDDM probands varies from 0.9% to almost 10% in different reports.⁵³⁻⁶⁰ In our studies, the frequency of ICA among all relatives averages 1.9%.⁵⁶ Siblings had the highest frequencies of ICA (brothers, 2.8%; sisters, 2.3%), whereas parents of both sexes have lower but equal frequencies at 1.7%. The significance of ICA in nondiabetic individuals appears to depend on age and/or relationship to the proband. Siblings and other ICA-positive individuals less than 20 years of age were more likely to develop IDDM.⁵⁶ Whether all individuals with ICA will develop IDDM awaits the outcome of these prospective studies as the pre-clinical phase may last a decade or longer.

In studies of organ-specific antibodies such as TMA, PCA, and ACA, approximately half of the patients have developed clinical disease of their

respective organs.²⁷⁻³³ We would expect, therefore, that only a proportion of individuals with ICA will develop IDDM. ICA-positive nondiabetic Addisonian patients have not developed IDDM at the same rate that ICA-positive siblings of IDDM probands have, despite the presence of high titers of ICA in these Addisonian patients. ICA have also been reported to disappear without the subsequent development of IDDM.¹⁰⁹⁻¹¹¹ The reason for the disappearance of ICA varies. Assay limitations such as observer bias of immunofluorescence from excessive background and differences in sensitivity of the pancreatic substrates are possible. In our tests, at least 5% of patients with ICA lose the autoantibodies from their sera upon follow-up without development of IDDM.¹¹² However, one such child with "disappearing" ICA on several samples after a positive result on an initial sample nonetheless developed IDDM. The titer of her ICA may have dropped below the detectable limits or disappeared prior to onset of IDDM. Other groups have reported higher rates of disappearance of ICA.^{110, 111} These same groups also find ICA at a higher frequency in families with IDDM probands.^{53, 54} Their assays might be more sensitive, at the expense of specificity (higher false positive rates). Complement-fixing ICA have been reported to be more predictive of IDDM than the standard assay for ICA.¹¹²⁻¹¹⁴ In our tests, the assay for complement-fixing ICA identifies the higher titered ICA.¹¹⁴ One group requires the presence of ICA in three serial samples of blood for the patient to be classified as positive.¹¹⁵

Identification of ICA in an individual without IDDM is associated with increased levels of anxiety.¹¹⁶ Although most have coped well after being told of the results, a high false positive rate from a very sensitive assay cannot be tolerated for a disease in which early diagnosis currently has little to no benefit. Once viable prevention of IDDM is achieved, however, then a high false positive rate does less harm and may be needed in order to identify most predisposed individuals.

Irvine and colleagues^{117, 118} in a study of type II patients found that the determination of ICA could identify IDDM prior to the onset of clinical disease and predict the need for insulin therapy. Thus, those patients who initially presented with the clinical features of type II diabetes and had ICA were likely to deteriorate to insulin dependence.^{117, 118} More recent studies have shown that insulin response after an intravenous (IV) glucose tolerance test may be a better predictor of eventual need for insulin.¹¹⁹ Whether ICA in gestational diabetes would predict those individuals who would eventually develop IDDM remains to be determined.¹²⁰ In our experience, ICA are rare in patients with clinically defined gestational diabetes. We have now followed three pregnancies in women who were ICA positive prior to conception. No glucose intolerance occurred during the pregnancies and none have developed IDDM to date.

Islet Cell Surface Antibodies

Within weeks of the report of ICA, ICSA reactive to the surface of a malignant human insulinoma cell line were described.²¹ Since that report, sev-

eral different cell substrates of animal origin have been used in the indirect immunofluorescent assay for ICSA.²² Another antibody to surface protein has been demonstrated by ³⁵S methionine labeling of rat islets and immunoprecipitation^{121, 122} of detergent extracts by patient sera. Although all of these antibodies are technically called ICSA, the different assays probably reflect antibodies to different β -cell antigens. The clinical usefulness of ICSA is limited by the difficulty of the assays, the lack of suitable human substrates, and the false positive rate of up to 15% in control subjects.^{22, 123}

Insulin Antibodies

Antibodies to endogenous insulin have been detected in newly diagnosed patients with IDDM even prior to the administration of insulin.²²⁻²⁶ These may be of prognostic value, particularly in younger patients. In one study, 30% of a selected group of nondiabetic individuals who later developed IDDM had INSAB.²⁶ In our studies, the presence of INSAB in a nondiabetic individual with ICA was associated with significantly lower insulin responses to intravenous glucose.²⁵ Thus, the presence of both ICA and INSAB appears to have an enhanced likelihood to develop IDDM. Whether INSAB alone could be of value to screen for IDDM is being tested in a prospective study. Considerable interest in the outcome of such studies exists, since the assay for INSAB lends itself to automation and mass screening.

Thyroid Microsomal Antibodies

The presence of TMA is diagnostic of thyroid autoimmune disease, such as Graves' or Hashimoto's disease. Graves' disease tends to occur before or at the onset of IDDM, whereas hypothyroidism is more likely to occur after the onset of IDDM.²⁷ As thyroid disease is a frequent occurrence in IDDM patients, particularly female patients, we have recommended that all patients with IDDM be tested for TMA.^{26, 27} Female siblings and mothers are also at significant risk for thyroid disease and should be evaluated, particularly if the proband has TMA or thyroid disease.^{35, 36} Those found to be positive should be followed with annual determinations of thyroid function studies.

Gastric Parietal Cell Antibodies

The presence of PCA are diagnostic of autoimmune atrophic gastritis and pernicious anemia.³¹ These diseases are also more common in IDDM patients and in their family members.^{35, 36} However, pediatricians are unlikely to see the sequelae of PCA in their patients with IDDM as these diseases tend to occur after 20 years of age. For PCA-positive older family members, the search for pernicious anemia is more rewarding.

Adrenocortical Antibodies

Schmidt's syndrome, the association of thyroid autoimmune disease and Addison's disease, was expanded to include IDDM.¹²⁴ Addison's disease is

more likely to occur in adult females with IDDM. Although adrenal insufficiency is rare in children, ACA can be found in 1.8% of children with IDDM who are at risk of developing Addison's disease.^{32, 33} The presence of ACA in an asymptomatic individual is occasionally associated with biochemical evidence of compensated adrenocortical insufficiency and with normal concentrations of cortisol and aldosterone but elevated plasma renin activity and ACTH levels.³³ In our experience, individuals with ACA and these biochemical abnormalities have subsequently developed Addison's disease. Routine screening for ACA in IDDM is probably not justified because of the low yield; however, adrenal insufficiency, if undiagnosed, might mimic diabetic ketoacidosis with a fatal outcome. But, screening of IDDM with TMA or thyroid disease would detect the majority of the patients at risk.³⁶

Cell-Mediated Immunity

The initial reports of cell-mediated immune defects in patients with established IDDM were done to explain the increased frequency of infections in IDDM.⁴⁰ The abnormalities found in the mixed lymphocyte culture or lectin stimulation assays were dependent on the metabolic state of the patient and not on the underlying immune process.⁴⁰ However, with the ability to identify the "prediabetic" individual using ICA, the role of cell-mediated immunity in IDDM can be studied without the confounding problems of the metabolic abnormalities.

Insulinitis and the destruction of pancreatic β cells are the pathologic hallmarks of IDDM.^{37, 38} The mononuclear (lymphocytic) infiltration of the pancreatic islets (insulitis) of newly diagnosed IDDM suggests that these cells are responsible for the β -cell destruction.³⁸ Immunofluorescent staining of these cells in the few pancreases so far able to be studied revealed that cytotoxic/suppressor T cells (CD8 positive) were the principal lymphocyte population although all cell types were present.³⁹ Increased expression of DR antigens is considered to be the sine qua non of T-cell activation.¹²⁵ The majority of the infiltrating lymphocytes expressed DR antigen and the IL-2 receptor, which indicates T-cell activation.⁴⁰ Increased expression of DR antigens but not IL-2 receptor were detected on the lymphocytes from four patients with recurrent insulitis following pancreatic transplantation.³⁹ The abnormal presence of activation antigens on peripheral blood T-lymphocytes has been reported in several immunologically mediated diseases, including IDDM.⁴¹⁻⁴⁵ Unfortunately, studies of the percentage of peripheral blood T cells expressing antigens of early activation in patients with newly diagnosed IDDM have been conflicting.⁴¹⁻⁴⁵ Because Janeway et al.¹²⁶ suggested that quantitative variations in DR antigen density on lymphocytes rather than the percentage of positive cells is central to the regulation of the immune response and autoimmunity, we determined the relative antigen density (RAD) of DR on the surface of peripheral blood T cells in our ICA-positive nondiabetic patients. Increased

RAD of DR was associated with ICA titer, the DR phenotype (DR3/DR4 heterozygotes having the highest density), and diminished insulin responses to IV glucose.⁴⁵ Thus, the presence of activated T cells occurred primarily in those individuals who were most likely to deteriorate to IDDM.⁴⁵ If the ongoing immunologic activity in the pancreatic islets is reflected in the peripheral blood, their determination would provide a means of monitoring intervention in the prediabetic phase.

Metabolic Abnormalities in "Prediabetes"

The end stage of the autoimmune destructive process in pancreatic islets is the loss of the ability to produce insulin. In longitudinal studies of twins and triplets, Soeldner and colleagues^{55, 60} demonstrated that the loss of the initial (first phase) insulin release after a bolus of IV glucose was the most predictive metabolic abnormality prior to the deterioration to clinical symptoms and insulin need. The first-phase insulin response is determined by summing the 1- and 3-minute insulin concentrations after the IV glucose. In nondiabetic ICA-positive patients, diminished first-phase insulin secretion to IV glucose appeared to be an ominous finding.^{55, 56, 59, 60} In our data, the first-phase insulin response to IV glucose was found to be significantly lower in ICA-positive relatives ($163 \pm 127 \mu\text{U/ml}$) than in ICA-negative relatives ($204 \pm 111 \mu\text{U/ml}$). Further, the insulin responses to IV glucose were significantly lower in ICA-positive siblings ($104 \pm 66 \mu\text{U/ml}$) than in the ICA-positive parents ($201 \pm 143 \mu\text{U/ml}$), consistent with our finding that siblings are more likely to develop IDDM. We have not found responses below $75 \mu\text{U/ml}$ in nondiabetic control persons. However, the first-phase insulin response to IV glucose, in our test, can be quite variable. Some of the variability may be due to the difficulties in the administration of the IV glucose, minor differences in the infusion rate, or variations in the state of hydration of the patient. In general, once the first-phase insulin response is below $75 \mu\text{U/ml}$, it rarely becomes normal again. Further study is needed to determine if there is a point beyond which the patient will not improve spontaneously; if so, intervention studies would be possible at that point.

Possible Prevention of Diabetes in Animal Models of IDDM and in Humans

Dietary restriction of protein in the "prediabetic" stage of BB/W rats prevented the development of diabetes in these genetically predisposed animals.⁹⁹ No formal dietary studies have been attempted in humans, although we have anecdotal experience that might suggest such an effect. Protein restriction was implemented in one of our prediabetic ICA-positive patients; but insulin secretion failed to improve.

Nicotinamide is used by two groups in ICA-positive nondiabetic individuals in an attempt to prevent the development of IDDM. The rationale for this approach arises from observations that pretreatment with nicotinamide prevents the diabetogenic effect of SZ.¹²⁶ One study from Japan has shown that nicotinamide treatments could prevent diabetes in NOD mice.¹²⁷

Another interesting approach was treatment of BB/W rats with insulin before the onset of clinical disease.¹²⁸ This therapy prevented the development of diabetes. This group of investigators used repeated blood transfusions as another means of preventing diabetes in the BB/W rat.¹³ These two studies were innovative in attempting to induce a state of immunologic tolerance to prevent diabetes. Other more traditional immunologic approaches such as thymectomy and immunosuppression by chemotherapy have also prevented diabetes in BB/W rats.¹³⁰⁻¹³² We have treated two adolescent girls who had ICA and falling insulin responses to IV glucose with azathioprine (Imuran). One gradually developed IDDM while being treated over 2 years, while the other has actually improved her insulin response on therapy.¹³³

If the underlying mechanism of the islet cell destruction in IDDM is an autoimmune disease, then suppressing the immune response is an obvious approach. During the past several years, a number of immunotherapies have been used in newly diagnosed IDDM patients.^{46-50, 134-138}

If antibodies such as ICA, INSAB, or ICSA were responsible for the islet cell damage, plasmapheresis might be beneficial. However, 11 newly diagnosed IDDM patients underwent plasmapheresis with no significant effect on glucose tolerance, although a transient decrease or loss of ICSA titers occurred.¹³⁴ When IDDM was thought to be of viral etiology, two newly diagnosed IDDM patients were treated with gamma interferon without detectable effect.¹³⁵ Corticosteroids at various doses had little or no effect on glucose tolerance although increased urinary excretion of C-peptide did occur in one study.^{47, 136, 137}

Several recent studies have demonstrated a beneficial effect of Imuran or cyclosporine in newly diagnosed IDDM patients.^{48-50, 138} These human studies had been preceded by reports from several groups using cyclosporine to prevent diabetes in BB rats.^{130, 138} The report by Stiller et al.⁴⁸ suggested a beneficial effect in that 50% of their patients given cyclosporine, within 6 weeks of diagnosis could be maintained insulin free for 1 year. Bach and colleagues⁴⁹ demonstrated increased β -cell function (C-peptide response to insulin secretagogues) and lowered or eliminated insulin requirements with cyclosporine treatment. The effect was dose dependent, requiring whole blood trough levels of cyclosporine approaching 300 ng/ml. Lack of weight loss at clinical onset of IDDM was associated with a favorable response to cyclosporine. The earlier the intervention, the more likely was a response to immunosuppressive therapy. The prediabetes screening studies using ICA may be the key to improving the effectiveness of this approach. The toxicity of cyclosporine has limited its use-

fulness. The most serious side effect is a dose-dependent nephrotoxicity with interstitial nephritis, fibrosis, glomerulosclerosis and tubular atrophy.¹³⁹ The presence of a therapeutic window may result in improvement in IDDM without inducing renal damage.

Harrison's group in Australia¹³⁸ has demonstrated prolongation of the remission period using Imuran in adults with newly diagnosed IDDM. We have used Imuran after induction with bolus pulses of methylprednisolone and high-dose prednisone.⁵⁰ Approximately 40% of the treated patients have a significantly prolonged remission.⁵⁰ Another group showed that intensified insulin therapy utilizing an artificial pancreas (Biostator®) totally suppressed endogenous insulin production for 2 weeks. This resulted in increased C peptide secretion and paradoxically continued insulin requirements.

All immunosuppressive therapies have several major disadvantages. The first is their oncogenic potential. Atypical B-cell lymphomas have been reported to be more common. This has been a major deterrent to their use in trials of newly diagnosed IDDM patients, as it impacts on the risk/benefit ratio. Second, all studies to date indicate that immunosuppressive therapy will be needed over the patient's lifetime. When cyclosporin A or Imuran was discontinued in patients who developed a diabetic remission, insulin dependence returned within 1 to 8 weeks. Patients with pancreatic transplants have demonstrated that immunosuppression suppresses but does not eliminate pancreatic islet cell immunoreactivity. Studies have shown that the immune system is resistant and even retains the ability to respond to β -cell antigens for as long as 20 years after diagnosis. This immunologic memory was demonstrated by the recurrence of insulinitis and IDDM in three sets of identical twins following pancreatic transplants.³⁸ Here, non-diabetic identical twin donor pancreas developed insulinitis, β -cell necrosis, and diabetes within weeks of transplantation.

In summary, IDDM is an autoimmune disease in which the preclinical phase of IDDM can be identified by various immunologic abnormalities, the most important being ICA. We are approaching the possibility of altering the natural history of IDDM in the prediabetic phase as the ability to more accurately predict impending disease continues to improve. Insulin replacement therapy may be replaced with some form of immune modulation for the prevention against IDDM.

References

1. Rosenbloom AL: Long term complications of type I (insulin dependent) diabetes mellitus. *Pediatr Ann* 1983; 12:655-683.
2. Cahill G, McDevitt H: Insulin dependent diabetes: The initial lesion. *N Engl J Med* 1981; 304:1451-1455.
3. Doniach D, Bottazzo GF, Cudworth AG, et al: Etiology of type 1 diabetes mellitus: Heterogeneity and immunological events leading to clinical onset. *Ann Rev Med* 1983; 34:13-21.

4. Eisenbarth GS: Type I diabetes mellitus: A chronic autoimmune disease. *N Engl J Med* 1986; 314:1360–1368.
5. Singal DP, Blajchman MA: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes* 1973; 22:429–432.
6. Nerup J, Platz P, Andersen OO, et al: HLA antigens and diabetes mellitus. *Lancet* 1974; 2:864–866.
7. Cudworth AC, Woodrow JC: HLA system and diabetes mellitus. *Diabetes* 1975; 24:345–349.
8. Cudworth AG, Wolf E: The genetic susceptibility of type I (insulin-dependent) diabetes mellitus. *Clin Endocrinol Metab* 1982; 11:389–408.
9. Rimoin DL, Rotter JI: The genetics of diabetes mellitus, in Andreani M, DiMario M, Federlin K, et al (eds): *Immunology of Diabetes*. Edinburgh, Krimpton Medical Press, 1984.
10. Maclaren N, Riley W, Skordis N, et al: Inherited susceptibility to insulin dependent diabetes is associated with HLA-DR1, while Dr2 and DR5 are protective. *Autoimmunity* 1988; in press.
11. Henson V, Maclaren NK, Winter WE, et al: Molecular genetics of insulin-dependent diabetes mellitus. *Mol Biol Med* 1986; 3:129–136.
12. Nepom BS, Palmer J, Kim SJ, et al: Specific genomic markers for the HLA-DQ subregion discriminate between DR4+ insulin-dependent diabetes mellitus and nDR4+ seropositive juvenile rheumatoid arthritis. *J Exp Med* 1986; 164:345–350.
13. Kim SJ, Holbeck SL, Nisperos B, et al: Identification of a polymorphic variant associated with HLAj-DQw3 and characterized by specific restriction sites within the DQ B-chain gene. *Proc Natl Acad Sci USA* 1985; 82:8139–8143.
14. Cohen-Haguenhauer O, Robbins E, Massart C, et al: Systematic study of HLA class II-B DNA restriction fragments in insulin-dependent diabetes. *Proc Natl Acad USA* 1985; 82:3335–3339.
15. Owerbach D, Hagglof B, Lernmark Å, et al: Susceptibility to insulin-dependent diabetes defined by restriction enzyme polymorphism of HLA-D region genomic DNA. *Diabetes* 1984; 33:958–965.
16. Henson V, Maclaren NK, Riley WJ, et al: Polymorphisms of DQβ genes in HLA-DR4 haplotypes from healthy and diabetic individuals. *Immunogenetics* 1987; 25(3):152–160.
17. Cohen D, Cohen O, Marcadet A, et al: Class II HLA-DC β-chain DNA restriction fragments differentiate among HLA-DR2 individuals in insulin-dependent diabetes and multiple sclerosis. *Proc Natl Acad Sci USA* 1984; 81:1774–1778.
18. Lendrum R, Walker G, Cudworth A, et al: Islet cell antibodies in diabetes mellitus. *Lancet* 1976; 2:1273–1276.
19. Bottazzo GF, Florin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 1974; 2:1279–1283.
20. Neufeld M, Maclaren NK, Riley WJ, et al: Islet cell and other organ specific antibodies in U.S. Caucasians and blacks with insulin dependent diabetes mellitus. *Diabetes* 1980; 29:589–594.
21. Maclaren N, Huang S, Fogh J: Antibody to cultured human insulinoma cells in insulin-dependent diabetes. *Lancet* 1975; 1:997–999.
22. Lernmark Å, Freedman Z, Hoffman C, et al: Islet cell surface antibodies in juvenile diabetes mellitus. *N Engl J Med* 1978; 299:375–378.

23. Palmer J, Asplin C, Clemons P, et al: Insulin antibodies in insulin dependent diabetics before insulin treatment. *Science* 1983; 222:1337-1339.
24. Wilkin T, Hoskins PJ, Armitage M, et al: Value of insulin autoantibodies as serum markers for insulin dependent diabetes mellitus. *Lancet* 1985; 1:480-482.
25. Atkinson MA, Maclaren NK, Riley WJ, et al: Are insulin autoantibodies markers for insulin dependent diabetes mellitus? *Diabetes* 1986; 35:894-898.
26. Srikanta S, Ricker AT, McCulloch DK, et al: Autoimmunity to insulin, beta cell dysfunction and development of insulin dependent diabetes mellitus. *Diabetes* 1986; 35:139-142.
27. Riley WJ, Maclaren NK, Lezotte DC, et al: Thyroid autoimmunity in insulin dependent diabetes mellitus: The case for routine screening. *J Pediatr* 1981; 9:350-354.
28. Riley WJ, Maclaren NK, Rosenbloom AR: Thyroid autoimmunity in young diabetics. *Lancet* 1982; 2:489-490.
29. Gilani BB, MacGillivray MH, Voorhess ML, et al: Thyroid hormone abnormalities at diagnosis of insulin dependent diabetes mellitus in children. *J Pediatr* 1984; 105:218-222.
30. Fraiser SD, Penny R, Snyder R, et al: Antithyroid antibodies in Hispanic patients with type 1 diabetes mellitus. *AJDC* 1986; 140:1278-1280.
31. Riley WJ, Toskes PP, Maclaren NK, et al: Predictive value of gastric parietal cell autoantibodies as a marker for gastric and hematologic abnormalities associated with insulin dependent diabetes. *Diabetes* 1982; 31:1051-1055.
32. Riley WJ, Maclaren N, Neufeld M: Adrenal autoantibodies and Addison's disease in insulin requiring diabetes. *J Pediatr* 1980; 97:191-195.
33. Ketchum K, Riley WJ, Maclaren N: Adrenal dysfunction in asymptomatic patients with adrenal antibodies. *J Clin Endocrinol Metab* 1984; 58:1166-1170.
34. Bright G, Blizzard R, Kaiser D, et al: Organ-specific autoantibodies in children with common endocrine diseases. *J Pediatr* 1982; 100:8-11.
35. Maclaren NK, Riley WJ: Thyroid, gastric, and adrenal autoimmunities associated with insulin-dependent diabetes mellitus. *Diabetes Care* 1985; 8(suppl 1):34-38.
36. Riley WJ: Associated autoimmune diseases in insulin-dependent diabetes mellitus, in Bergman M (ed): *Principles of Diabetes Management*. New York, Elsevier, 1986.
37. Gepts W: Pathological anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 1965; 14:619-623.
38. Sibley RK, Sutherland DER, Goetz F, et al: Recurrent diabetes mellitus in the pancreas iso- and allograft. *Lab Invest* 1985; 53:132-144.
39. Bottazzo GF, Dean BM, McNally JM, et al: In-situ characterization of autoimmune phenomena and expression of HLA molecules in diabetic insulinitis. *N Engl J Med* 1985; 313:353-360.
40. Gupta S: Lymphocyte response in diabetes mellitus, in Gupta Sudhir (ed): *Immunology of Clinical Experimental Diabetes*. New York, Plenum Medical Book Co, 1984, pp 329-349.
41. Jackson R, Morris M, Haynes G, et al: Increased circulating Ia antigen bearing T cells in type I diabetes mellitus. *N Engl J Med* 1982; 306:785-787.
42. Pozzili P, Zuccarini O, Iavicoli M, et al: Monoclonal antibodies defined abnormalities of T-lymphocytes in tpe I (insulin-dependent) diabetes. *Diabetes* 1983; 32:91-92.

43. Alviggi L, Hoskins PJ, Pyke DA, et al: Pathogenesis of insulin dependent diabetes: A role for activated T lymphocytes. *Lancet* 1984; 1:4-6.
44. Hayward AR, Herberger M: Culture and phenotype of activated T-cells from patients with type I diabetes mellitus. *Diabetes* 1984; 33:319-323.
45. Hitchcock CL, Riley WJ, Alamo A, et al: Lymphocyte subsets and activation in prediabetes. *Diabetes* 1986; 35:1416-1422.
46. Winter WE, Maclaren NK: Type insulin dependent diabetes: An autoimmune disease that can be arrested or prevented with immunotherapy? in Barness LA (ed): *Advances in Pediatrics*. Chicago, Year Book Medical Publishers, 1985, pp 159-175.
47. Elliot RB, Crossly JR, Berryman CC, et al: Partial preservation of pancreatic B-cell function in children with diabetes. *Lancet* 1981; 2:1-3.
48. Stiller CR, Laupacis A, Dupre J, et al: Cyclosporine for treatment of early type I diabetes: Preliminary results. *N Engl J Med* 1983; 308:1226-1227.
49. Feutren G, Papoz L, Assan R, et al: Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset: Results of a multicentre double-blind trial. *Lancet* 1986; 2:119-124.
50. Maclaren NK, Silverstein JH, Spillar RP, et al: Immunosuppressive therapy in insulin dependent diabetes. *Acta Paediatr Japonica* 1987; 29:349-354.
51. Maclaren NK, Elder ME, Robbins VW, et al: Autoimmune diathesis and T lymphocyte immunoincompetence in BB rats. *Metabolism* 1983; 32:92-96.
52. Leiter EH, Prochazka M, Coleman DL: The non-obese (NOD) mouse. *Am J Pathol* 1987; 128(2):380-383.
53. Gorsuch AN, Lister J, Dean BM, et al: Evidence for a long prediabetic period in type 1 (insulin-dependent) diabetes mellitus. *Lancet* 1981; 2:1363-1365.
54. Betterle C, Lanette F, Tiengo A, et al: Five year followup of nondiabetics with islet cell antibodies. *Lancet* 1982; 1:284-285.
55. Srikanta A, Ganda OP, Rabizadeh A, et al: First degree relatives of patients with type I diabetes: Islet cell antibodies and abnormal insulin secretion. *N Engl J Med* 1985; 313:461-464.
56. Riley WJ, Spillar R, Waltz J, et al: Predictive value of islet cell autoantibodies: 6 years' experience. *Diabetes* 1984; 33(suppl 1):44A.
57. Bruining GJ, Molenaar JL, de Jongh BM, et al: Prediction of type I diabetes mellitus: A report on three cases. *Eur J Paediatr* 1985; 143:175-178.
58. Ginsberg-Fellner F, Witt ME, Franklin BH, et al: Triad of markers for identifying children at risk of developing insulin-dependent diabetes. *JAMA* 1985; 254:1469-1472.
59. Srikanta A, Ganda OP, Jackson RA, et al: Islet cell antibodies and beta cell function in monozygotic triplets and twins initially discordant for type I diabetes mellitus. *N Engl J Med* 1985; 308:322-325.
60. Srikanta A, Ganda DP, Jackson RA, et al: Type I diabetes in monozygotic twins: Chronic progressive beta cell dysfunction. *Ann Int Med* 1983; 99:320-326.
61. Tattersall RB, Pyke DA: Genetic susceptibility in diabetic identical twins. *Lancet* 1973; 22:355-357.
62. Warram JH, Krolewski AS, Gottleib MS, et al: Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N Engl J Med* 1984; 311:149-152.
63. Rotter J, Riley WJ, Spillar R, et al: The male predisposition for insulin dependent diabetes. *Diabetes* 1984; 33(suppl 1):37A.

64. Dahlquist G, Gustavsson KH, Holmgren G, et al: The incidence of diabetes mellitus in Swedish children 0–14 years of age: A prospective study 1977–1980. *Acta Paediatr Scand* 1982; 71:7–14.
65. Irvine WJ: Classification of idiopathic diabetes. *Lancet* 1978; 1:638.
66. Vadheim CM, Rotter J, Maclaren NK, et al: Preferential transmission of diabetes alleles within the HLA gene complex. *N Engl J Med* 315:1314–1318.
67. Riley WJ, Glancey L, Phinney R: Thyro-gastric autoimmune genes: Another risk factor for insulin dependent diabetes? *Diabetes* 1982; 31:45A.
68. Gorsuch A, Dean B, Bottazzo GF, et al: Thyrogastric autoimmunity in type 1 diabetes. *Br Med J* 1980; 1:145–147.
69. Bell GI, Horita S, Karam JH: A highly polymorphic locus near the human insulin gene is associated with insulin dependent diabetes mellitus. *Diabetes* 1984; 33:176–183.
70. Raffel LJ, Vadheim CM, Klein R, et al: HLA and the insulin gene polymorphism in IDD: Is there evidence for interaction? *Diabetes* 1985; 34:500A.
71. Farid NR, Newton RM, Noel EP, et al: The operation of immunological networks in Graves' disease. *Tissue Antigens* 1978; 12:205–211.
72. Whittingham S, Mathews JP, Schonfield MS, et al: Interactions of HLA and Gm in autoimmune chronic active hepatitis. *Clin Exp Immunol* 1981; 43:80–86.
73. Tait BD, Propert DN, Harrison LC, et al: Interaction between HLA antigens and immunoglobulin (Gm) allotypes in susceptibility to type 1 diabetes. *Tissue Antigens* 1986; 27(5):249–255.
74. Rich SS, Weitkamp LR, Guttormsen S, et al: Gm, Km, and HLA in insulin-dependent type 1 diabetes mellitus. *Diabetes* 1986; 35:927–932.
75. Chappel CI, Chappel WR: The discovery and development of the BB rat colony. *Metabolism* 1983; 32(suppl 1):8–20.
76. Katooka S, Stoh J, Fujiya H, et al: Immunologic aspects of nonobese (NOD) mouse. *Diabetes* 1983; 32:247–251.
77. Rossini AA, Williams RM, Mordes JP, et al: Spontaneous diabetes in the gnotobiotic BB/W rat. *Diabetes* 1979; 28:1031–1033.
78. Craighead JE, McLane MF: Diabetes mellitus: Introduction in mice by encephalomyocarditis virus. *Science* 1968; 162:913–914.
79. Webb SR, Loria RM, Madge GE, et al: Susceptibility of mice to group coxsackie B virus is influenced by the diabetic gene. *J Exp Med* 1976; 143:1239–1248.
80. Adams SF: The seasonal variation in the onset of acute diabetes. *Arch Intern Med* 1926; 37:861–864.
81. Gamble DR, Taylor KW: Seasonal incidence of diabetes mellitus. *Br Med J* 1969; 3:631–633.
82. Yoon JW, Onodera T, Notkins AL: Virus-induced diabetes mellitus VIII: Passage of encephalomyocarditis virus and severity of diabetes in susceptibility and resistant strains of mice. *J Gen Virol* 1977; 37:225–232.
83. Gamble DR, Taylor KW: Coxsackie B virus and diabetes. *Br Med J* 1973; 1:289–290.
84. Riley WJ, Maclaren NK, Rand K, et al: Inherited autoimmunity versus coxsackie B4 in insulin dependent diabetes. *Diabetes* 1980; 29(suppl 2):53A.
85. Banatvala JE, Bryant J, Schernthaner G, et al: Coxsackie B, mumps, rubella, and cytomegalovirus specific IgM responses in patients with juvenile onset insulin dependent diabetes mellitus in Britain, Austria, and Australia. *Lancet* 1985; 2:1409–1412.

86. Yoon J-W, Austin M, Onodera T, et al: Virus induced diabetes mellitus. *N Engl J Med* 1979; 300:1173-1176.
87. Asplin MS, Cooney MK, Crossley JR, et al: Coxsackie B4 infection and islet cell antibodies three years before overt diabetes. *J Pediatr* 1982; 101:398-400.
88. Mertens TH, Gruneklee K, Eggers HJ: Neutralizing antibodies against coxsackie B viruses in patients with recent onset of type 1 diabetes. *Eur J Pediatr* 1983; 140:293-294.
89. Sultz HA, Hart BA, Zielozny M: Is mumps virus an etiologic factor in juvenile diabetes? *J Pediatr* 86:654-656.
90. Helmke K, Otten A, Willems W: Islet antibodies in children with mumps infection. *Lancet* 1980; 2:211.
91. Menser M, Forest J, Bransky R: Rubella infection and diabetes mellitus. *Lancet* 1978; 1:57-60.
92. Clarke WL, Shaver KA, Bright GM, et al: Autoimmunity in congenital rubella syndrome. *J Pediatr* 1984; 104:370-373.
93. Grodsky SM, Anderson CE, Coleman DL, et al: Metabolic and underlying causes of diabetes mellitus. *Diabetes* 1982; 31:45-53.
94. Riley WJ, McConnel T, Maclaren NK, et al: The diabetogenic effects of streptozotocin in mice are prolonged and inversely related to age. *Diabetes* 1981; 30:718-723.
95. Bonnenie-Nielsen V, Steffes MW, Lernmark Å: A major loss in islet mass and B-cell function precedes hyperglycemia in mice given multiple low doses of streptozotocin. *Diabetes* 1981; 30:424-429.
96. Karam JH, Lewitt PA, Young CW, et al: Insulinopenic diabetes after rodenticide (Vacor) ingestion: A unique model of acquired diabetes in man. *Diabetes* 1980; 29:971-978.
97. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, et al: Relationship between breast feeding and incidence of insulin-dependent diabetes mellitus: A hypothesis. *Lancet* 1984; 2:1083-1088.
98. Skordis N, Atkinson M, Beppu H, et al: The effect of diet on incidence and time of onset of insulin dependent diabetes in the BB rat. *Immunology of Diabetes: 6th International Congress of Immunology* 1986; 717:319-324.
99. Scott FW: Alterations in single diet constituents and diabetes expression in the BB rat. *Immunology of Diabetes: 6th International Congress of Immunology* 1986; 717:307-312.
100. Elliott RB, Martin JM: Dietary protein: A trigger of insulin dependent diabetes in the BB rat? *Diabetologia* 1984; 26:297-299.
101. Helgason T, Jonasson MR: Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet* 1981; 2:716-720.
102. Doberson MJ, Bell AM, Jensen AB, et al: Detection of antibodies to islet cells and insulin with paraffin-embedded pancreas as antigen. *Lancet* 1979; 2:1078.
103. Srikanta S, Rabizadeh A, Omar MAK, et al: Assay for islet cell antibodies protein A-monoclonal antibody method. *Diabetes* 1984; 34:300-305.
104. Kobayashi T, Sugimoto T, Itoh T, et al: The prevalence of islet cell antibodies in Japanese insulin-dependent and non-insulin dependent diabetic patients studied by indirect immunofluorescence and by a new method. *Diabetes* 1986; 35:335-340.
105. Yagihashi S, Suzuki H, Doberson MJ, et al: Autoantibodies to islet cells: Comparison of methods. *Lancet* 1982; 2:1218.

106. Bottazzo GF, Gleichman H: Immunology and diabetes workshops: Report of the first international workshop on the standardization of cytoplasmic islet cell antibodies. *Diabetologia* 1986; 29:125-126.
107. Gleichman H, Bottazzo GF: Progress toward standardization of cytoplasmic islet cell-antibody assay. *Diabetes* 1987; 36:578-584.
108. Schatz DA, Barrett DJ, Maclaren NK, et al: Polyclonal nature of islet cell antibodies in insulin dependent diabetes. *Autoimmunity* 1988; in press.
109. Mutch WJ, Stowers JM: Fluctuating islet-cell antibodies. *Lancet* 1984; 1:1019.
110. Spencer KM, Dean BM, Tarn A, et al: Fluctuating islet-cell autoimmunity in unaffected relatives of patients with insulin-dependent diabetes. *Lancet* 1984; 1:764-766.
111. Riley WJ, Maclaren NK: Islet cell antibodies are seldom transient. *Lancet* 1984; 1:1351-1352.
112. Bosi E, Becker F, Schwarz GH, et al: Natural history of pre-type 1 diabetes in polyendocrine patients with islet cell antibodies. *Diabetes* 1987; 36(suppl 1):64A.
113. Bottazzo GF, Gorsuch AN, Dean BM, et al: Complement-fixing islet-cell antibodies in type-I diabetes: Possible monitors of active beta-cell damage. *Lancet* 1980; 1:668-672.
114. Riley WJ, Neufeld M, Maclaren N: Complement fixing islet cell antibodies: A separate species? *Lancet* 1980; 2:113-116.
115. Tarn AC, Thomas JM, Howard W, et al: 9 years followup in a family study: The risks for insulin dependent diabetes (IDDM). *Diabetes* 1987; 36(suppl 1):72A.
116. Johnson SB, Hansen CA, Nurick M: Will I get diabetes? The psychological impact of ICA screening? *Diabetes* 1987; 36(suppl 1):72A.
117. Irvine WJ, McCallum CJ, Gray RS, et al: Clinical and pathogenic significance of islet-cell antibodies in diabetics treated with oral hypoglycemia agents. *Lancet* 1977; 1:1025-1027.
118. Irvine WJ, Gray RS, McCallum CJ: Pancreatic islet-cell antibody as a marker for asymptomatic and latent diabetes and prediabetes. *Lancet* 1976; 2:1097-1102.
119. Scott RS, Mason DR, Iris FJ, et al: Insulin deficiency in non-insulin dependent diabetics. *Diab Res Clin Pract* 1986; 2(6):359-364.
120. Rubinstein P, Walkerr M, Krassner J, et al: HLA antigens and islet cell antibodies in gestational diabetes. *Diabetes* 1981; 3:271-275.
121. Baekkeskov S, Nielsen JH, Marner B, et al: Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 1982; 298:167-169.
122. Baekkeskov S, Dyberg T, Lernmark A: Autoantibodies to a 64-kilodalton islet cell protein precede the onset of spontaneous diabetes in the BB rat. *Science* 1984; 224:1348-1350.
123. Toguchi Y, Ginsberg-Fellner F, Rubenstein P: Cytotoxic islet cell surface antibodies (ICSA) in patients with type I diabetes and their first degree relatives. *Diabetes* 1985; 34:855-860.
124. Schmidt MB: Eine biglandulare Erkrankung (Nebennieren und Schidddrüse) bei morbus Addisonii. *Verh Dtsch Ges Pathol* 1926; 21:212-221.
125. McNicholas JM, Murphy DB, Matis LA, et al: Immune response gene function correlates with the expression of an Ia antigen. *J Exp Med* 1982; 155:490-507.

126. Janeway CA, Bottomly K, Babich J, et al: Quantitative variation in Ia antigen expression plays a central role in immune regulation. *Immunol Today* 1984; 5:99-105.
127. Yamada K, Nonaka K, Hanafusa T, et al: Preventive and therapeutic effects of large dose nicotinamide injections on diabetes associated with insulinitis. *Diabetes* 1982; 31:749-753.
128. Gottfredsen CF, Buschard K, Frandsen EK: Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes-prone animals. *Diabetologia* 1985; 28:933-935.
129. Rossini AA, Mordes JP, Pelletier AM, et al: Transfusion of whole blood prevent spontaneous diabetes mellitus in the BB/W rat. *Science* 1983; 219:975-977.
130. Like AA, Dirodi V, Thomas A, et al: Prevention of diabetes mellitus in the BB/W rat with cyclosporin A. *Am J Pathol* 1984; 117:92-97.
131. Like AA, Kislauskis E, Williams RM, et al: Neonatal thymectomy prevents spontaneous diabetes in the BB/w rat. *Science* 1982; 216:644-646.
132. Naji A, Silvers WK, Bellgrau D, et al: Spontaneous diabetes in rats: Destruction of islets is prevented by immunologic tolerance. *Science* 1981; 213:1390-1392.
133. Riley WJ, Maclaren NK, Spillar RS: Reversal of deteriorating glucose tolerance with azathioprine in prediabetes. *Transplan Proc* 1986; 18:819-822.
134. Ludvigsson J, Heding L, Lieden G, et al: Plasmapheresis in the initial treatment of insulin-dependent diabetes mellitus in children. *Br J Med* 1983; 286:333-335.
135. Rand KH, Rosenbloom AL, Maclaren NK, et al: Human leukocyte interferon treatment of two children with insulin dependent diabetes. *Diabetologia* 1981; 21:116-120.
136. Elliot RB, Crossly JR, Berryman CC, et al: Partial preservation of pancreatic B-cell function in children with diabetes. *Lancet* 1981; 2:1-3.
137. Sotos JF, Romshe CA, Zipf WB: Immunosuppressive "pulse" treatment in IDD of recent onset. *Bull Inter Study Group Diab Child Adol* 1984; 10:19.
138. Harrison LC, Colman PG, Dean B, et al: Increase in remission rate in newly diagnosed type 1 diabetic subjects treated with azathioprine. *Diabetes* 34:1306-1308.
139. Myers BB, Ross J, Newton L, et al: Cyclosporine-associated chronic nephropathy. *N Engl J Med* 1984; 311:699-705.
140. Shah SC, Malone JJ, Simpson NE: Suppression of endogenous insulin secretion in new onset insulin dependent diabetics prolongs beta cell function. *Diabetes* 1987; 36:61A.

Adverse Reactions to Foods and Their Relationship to Skin Diseases in Children*

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Adverse reactions to foods have been implicated in the causation of many clinical problems ranging from life-threatening anaphylaxis to tension-fatigue syndrome. Indeed, the literature is replete with both documented and anecdotal accounts of symptoms attributed to food ingestion. Unfortunately, while some adverse responses such as anaphylactic (immunoglobulin E [IgE]-mediated) reactions have been systematically studied,¹⁻⁶ the pathogenic role of food (if any) in other responses such as tension-fatigue syndrome, hyperkinesia, and others is still unresolved. This chapter reviews present knowledge regarding the relationships between adverse reactions to foods and the pathogenesis of three skin disorders in children: urticaria, atopic dermatitis, and dermatitis herpetiformis (DH).

Terminology

"Food allergy" has been used as a generic term by both physicians and patients to refer to many different types of clinical reactions and has resulted in confusion in terminology. To standardize nomenclature, the American Academy of Allergy and Immunology Committee on Adverse Reactions to Foods has suggested that the following definitions be used in discussing aspects of food-induced reactions.⁶

Adverse reaction to a food: Clinically abnormal response believed caused by an ingested food or food additive.

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Food hypersensitivity (allergy): Immunologic reaction resulting from the ingestion of a food or food additive.

Food anaphylaxis: Classic allergic hypersensitivity reaction to food or food additives involving IgE antibody and release of chemical mediators.

Food idiosyncrasy: Quantitatively abnormal response to a food or food additive; response differs from its physiologic or pharmacologic effect and resembles a hypersensitivity reaction but does not involve an immune mechanism; an anaphylaxis-like reaction may be called "anaphylactoid."

Food intolerance: General term describing an abnormal physiologic response to an ingested food or food additive that is not proved to be immunologic in nature; category includes idiosyncratic, pharmacologic, metabolic, or toxic responses to food or food additives.

Food toxicity (poisoning): Term implying an adverse effect caused by direct action of a food or food additive on host recipient without the involvement of immune mechanisms; nonimmune release of chemical mediators may take place; toxins may be either from the food itself or from microorganisms; anaphylaxis-like reactions may be called "anaphylactoid."

Anaphylactoid reaction to a food: Anaphylaxis-like reaction to a food or food additive as a result of nonimmune release of chemical mediators.

Pharmacologic food reaction: Adverse reactions to a food or food additive as a result of a naturally derived or added chemical that produces a drug-like or pharmacologic effect in the host.

Metabolic food reaction: Adverse reactions to a food or food additive as the result of the effect of the substance on the metabolism of the host recipient.

As will be discussed, evidence indicates that foods may induce urticaria, atopic dermatitis, and DH through immunologic mechanisms. Thus, these conditions can be considered, at least in part, to be manifestations of food allergy or hypersensitivity in children.

Immunobiology of the Gastrointestinal Tract and Skin

To understand how foods can induce cutaneous manifestations through immunologic mechanisms, an understanding of the major immunologic elements of both the gastrointestinal tract and the skin is essential.

Gastrointestinal Tract

Food molecules enter the intestinal epithelial cells in vacuoles following invagination of the plasma membrane. These vacuoles migrate centrally, coalesce, and merge with lysosomes. Most of the internalized protein is digested by lysosomal enzymes, but a small amount is extravasated into the basilar interstitial space, from which it can cross the basement membrane into the lamina propria.⁷ Within the lamina propria, food antigens can interact with various lymphocytes, macrophages, or mast cells.

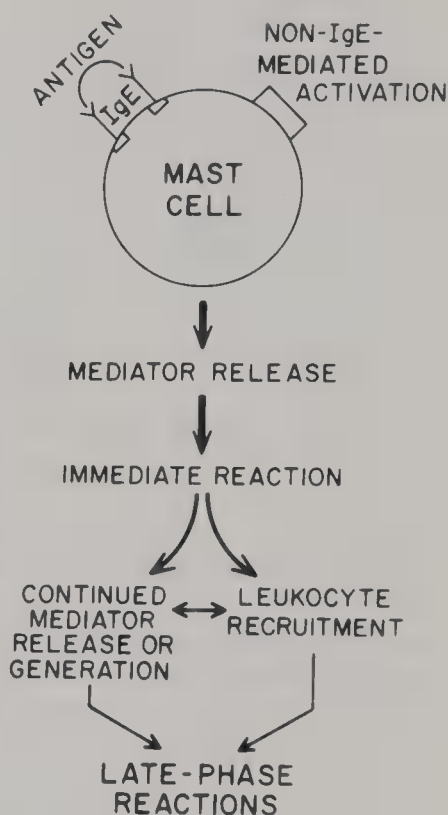
Alternatively, food molecules may interact with immunocompetent cells in Peyer's patches. Peyer's patches are collections of lymphoid tissue that are prevented from exposure to the intestinal lumen by a layer of epithelial cells, termed M (membranous) cells, which differ morphologically from other intestinal epithelial cells. Peyer's patches contain both T- and B-lymphocytes. It appears that both T- and B-lymphocytes, following antigen exposure within the Peyer's patch, migrate into the lymphatic system, enter in turn the thoracic duct and the systemic circulation, and eventually return to the lamina propria within the gastrointestinal tract.

The humoral immune system of the gastrointestinal tract includes the local production of immunoglobulins A (IgA) and M (IgM). In adults, IgA-producing cells predominate, with IgM-producing cells the next most common (6% to 18%). IgG-producing (3% to 4%) and IgE-producing and IgD-producing (<1%) cells can also be found.^{8, 9} Measurement of fecal IgA antibodies in bottle-fed, healthy infants suggests that the intestinal IgA-producing cell system may reach maturity as early as 1 to 2 months of age.¹⁰ It is felt that gut-associated immunoglobulins function in the defense against microorganisms and control of antigen absorption locally within the gut. In addition, antigen-specific antibody could combine with absorbed antigens, resulting in circulating immune complexes. IgE antibody, once formed, would bind to tissue mast cells or circulating basophils and, as discussed later, trigger mediator release following contact with antigen.

Cell-mediated immunity involving activated T cells and macrophages can also be a factor both locally within the gut or distally following the circulation and translocation of antigen-primed T cells that originate within Peyer's patches. Once activated, T cells release lymphokines that can both initiate and propagate immune defense mechanisms as well as hypersensitivity and inflammatory responses.

An important limb of the allergic response to food antigens involves IgE antibody, mast cells and basophils, mast cell or basophil mediator release, and the biologic effects of these mediators on various target organs. It is known that certain individuals are genetically "programmed" to preferentially produce IgE antibody responses following inhalation or ingestion of various substances. Once antigen-specific IgE antibody formation has occurred, the IgE antibody binds with high affinity to membrane receptors located on the surface of both tissue mast cells and circulating basophils. Mast cell or basophil mediator release occurs when antigen bridges two IgE molecules (hence, two IgE receptors) (Fig 1).

In addition to IgE-dependent mediator release, mast cell and basophil secretion can be induced by various factors that appear to bypass IgE and act directly on the membrane surface (Table 1). Such non-IgE-dependent triggering factors include complement fragments (C3a, C5a, and C4a, which act as anaphylatoxins); neuropeptides; neutrophil proteins; eosinophil proteins (e.g., major basic protein)¹¹; histamine-releasing factors from mononuclear cells^{12, 13} and platelets¹⁴; and various pharmaceuticals including succinylcholine, vancomycin, morphine and codeine, among oth-

**FIG 1.**

Mast cell mediator release can be initiated both by antigen binding of at least two IgE antibody molecules or through direct mast cell activation by a variety of substances. Once mediator release occurs, immediate (minutes) allergic reactions develop followed by late-phase allergic reactions (hours). The precise pathogenesis of the late response is still unknown but may involve leukocyte recruitment to areas of mast cell degranulation with additional inflammatory mediator release by these various cell types themselves (eosinophils, neutrophils, macrophages, lymphocytes, basophils) or through additional interaction with resident mast cells.

ers. Thus, various substances, including foods, could conceivably trigger anaphylactoid reactions (non IgE dependent) through complement activation, direct effects on tissue mast cells, or through the induction of tissue inflammation (involving various leukocytes including neutrophils, eosinophils, lymphocytes, and platelets) resulting from other immunologic mechanisms such as immune complex formation or T cell activation.

Mast cells have been observed in gastrointestinal tissue as early as the third fetal month and gradually increase in numbers during infancy and childhood until adult levels are reached.¹⁵ The skin and gastrointestinal tract mucosa are among the tissues particularly rich in mast cells. Normal human skin contains, on average, 7,000 mast cells/cu mm,¹⁶ and human duodenal mucosa contains approximately 20,000. In the gastrointestinal tract, mast cells occur in the epithelium, mucosa, submucosa, muscular layers, and serosa.¹⁷ Mast cells within the gastrointestinal tract layers vary in distribution. In general, mast cells in the mucosa occur randomly throughout the lamina propria. In the duodenal mucosa, they are especially prominent in the loose connective tissue in and around Brunner's glands. Mast cells in the submucosa, muscular layer, and serosa occur both in approximation to small blood vessels and at sites distant from these vessels. The number of cells within the mucosa varies considerably, depending on the level of the gastrointestinal tract.¹⁸ In rodents, mast cells

TABLE 1.
Agents Capable of Triggering Non-IgE-
Dependent Mast Cell Mediator Release

Endogenous

Complement fragments (C5a > C3a > C4a)
 Neuropeptides (substance P, somatostatin)
 Adenosine (potentiates secretion)
 Neutrophil lysosomal proteins
 Eosinophil major basic protein
 Lymphocyte and monocyte histamine releasing factors
 Interleukin 1

Exogenous

Anesthetic agents (succinylcholine, d-tubocurarine)
 Antibiotics (polymixin B, vancomycin)
 Narcotics (morphine, codeine)
 Iodinated contrast material
 Nonisotonic solutions
 ? Foods (strawberries)

located within the gastrointestinal tract appear to be heterogenous both morphologically (based on their ability to withstand formalin fixation) and functionally (in response to various secretagogues and pharmacologic modulation of secretion).¹⁹ In humans, gastrointestinal mast cell heterogeneity can be demonstrated morphologically²⁰ and by neutral protease composition,²¹ but it remains to be established if functional differences between these various populations also exist. Recent work by Irani et al.²¹ suggests that the population density of certain mast cell populations within the gut may be influenced by T-lymphocyte functional responses. Human intestinal mast cells release histamine in response to anti-IgE antibody and calcium ionophores, but not to compound 48/40 or f-methionyl peptide.^{21, 22} In addition, they generate both prostaglandin D₂ and leukotriene C₄.²²

Mast cells can release or generate a variety of mediators with diverse biologic effects (Tables 2 through 4).²³ Preformed mediators consist of those molecules that are rapidly released into the local tissue fluid (see Table 2) and those that remain more tightly bound to heparin, which serves as the "backbone" to which various granule mediators are attached through chemical interactions (see Table 3). Mediators are also generated by both mast cells and surrounding tissue cells (see Table 4). Within the gut, mast cell mediators could induce tissue inflammation and mucous secretion.^{4, 5} Perhaps more importantly, in terms of triggering distal cuta-

TABLE 2.
Mast Cell Granule Mediators That Are Preformed and Rapidly Released Into the Surrounding Tissues

Mediator	Structure	Biologic Effects
Histamine	β -imidazolyl ethylamine	H1: Smooth muscle contractions; increased vascular permeability; pruritus; irritant receptor stimulation; prostaglandin generation; increases in cGMP H2: Gastric acid secretion; increases in cAMP and mucous secretion; inhibition of basophil histamine release, lymphocyte lymphokine release, and neutrophil lysosomal enzyme release; inhibition of eosinophil migration and T cell-mediated cytotoxicity H1 + H2: Vasodilation (hypotension); flush; headache
Eosinophil chemotactic factors of anaphylaxis (ECF-A)	Val/Ala-gly-ser-glu MW = 360 – 390	Chemotactic attraction and deactivation of eosinophils and neutrophils
Neutrophil chemotactic factor of anaphylaxis (NCF-A)	Neutral protein MW = 400,000 – 600,000	Preferential attraction and deactivation of neutrophils
Exoglycosidases β -glucuronidase β -hexosaminidase β -galactosidase	Proteins	Cleave carbohydrate residues
Arylsulfatase A	Glycoproteins	Hydrolysis of sulfates
Kininogenases	Proteins	Generates kinins that cause bronchoconstriction and vasodilation

neous manifestations, is the effects of mediators on enhancing bowel wall and vascular permeability thereby potentially facilitating the absorption of various food macromolecules.

Skin

In considering the skin as an immunologic organ, one has to include the following elements: dermal-epidermal junction (DEJ), Langerhans' cells, indeterminate epidermal cells, epidermal cytokines, and mast cells. The DEJ serves three main functions.²⁴ First, it provides a permeability barrier between the vascular dermis and avascular epidermis. Soluble factors in the plasma and vasculature must pass through two basement membranes, one around the dermal blood vessels and one within the DEJ, before reaching the epidermal cells. Negatively charged anionic sites within the basement membrane may act as "pores" in the membrane and selectively

TABLE 3.
Preformed Mediators That Form Strong Chemical Bonds With
the Mast Cell Granule Heparin Matrix

Mediator	Structure	Biologic Effects
Heparin	Proteoglycan MW = 650,000	Antithrombin activity, anticoagulation, anticomplement activity
Chymase/tryptase	Proteins MW = 25,000– 30,000	Proteolysis
Peroxidase	Protein	Cleavage of H ₂ O ₂
Arylsulfatase B	Glycoprotein	Cleave sulfate esters
Inflammatory factor of anaphylaxis (IF- A)	Oligopeptide MW = 1,400	Chemoattraction of neutrophils

inhibit or allow passage of soluble substances.²⁵ Second, the contact between the epidermal cells and macromolecules within the DEJ may influence the biology of the epidermal cell by "cell-matrix interactions." Thus, constituents within the basement membrane may influence keratinocyte involvement in wound healing, organogenesis, tissue turnover, and maintenance.²⁴ Third, the DEJ is important for adherence of the epidermis to the underlying dermal connective tissue. This function of DEJ has clinical importance when considering subepidermal blistering diseases of the skin.

The importance of Langerhans' cells, indeterminate cells, and epidermal cytokines has been recently reviewed.^{26, 27} Langerhans' cells compose 2% to 8% of epidermal cells. They are derived from the bone marrow, undergo gradual turnover, are highly dendritic, and identified by their possession of a distinctive intracytoplasmic organelle known as the Langerhans' cell granule. This granule is a trilaminar structure sometimes containing a bulb at one end; its function is unknown. Cells within the epidermis that have the ultrastructural features of Langerhans' cells but lack the characteristic granule are termed indeterminate cells. In normal epidermis, Langerhans' and indeterminate cells are the only cells that express immune response-associated (Ia, HLA-DR) antigens. Langerhans' cells also express Fc and C3 receptors on their membrane surface. IgE receptors on these cells have also been described.²⁸ Langerhans' cells induce allogeneic and antigen-specific T-cell activation and are required for epidermal cell-induced generation of cytotoxic T-lymphocytes. By virtue of their antigen-presenting capacity, Langerhans' cells probably contribute to

TABLE 4.
Mediators Generated by Mast Cells

Mediator	Cell Source or Pathway	Biologic Effects
Prostaglandin E	Cyclooxygenase pathway of arachidonic acid metabolism	Vasopermeability; decreases mediator release
Prostaglandin D ₂	Major cyclooxygenase pathway product produced by mast cells (not by basophils)	Vasopermeability; chemoattraction of leukocytes; smooth muscle contraction
Prostaglandin F ₂	Cyclooxygenase pathway	Smooth muscle contraction; enhances mucous secretion
Leukotriene B ₄	Lipoxygenase pathway product of arachidonic acid metabolism; predominantly made by neutrophils	Neutrophil chemotaxis
Leukotriene C ₄ , D ₄ , E ₄	Lipoxygenase pathway; made by both eosinophils and mast cells	Vasopermeability; smooth muscle contraction; enhance mucous secretion; tissue inflammation
Hydroxyeicosatetraenoic acid derivatives	Quantity and type dependent on cell source	Chemotaxis; mucous secretion
Platelet activating factor	Quantity produced dependent on cell source	Vasopermeability; cutaneous vasoconstriction; platelet, neutrophil and monocyte aggregation

contact sensitization and immunosurveillance against cutaneous viral infection and neoplasia.²⁷

Epidermal cytokines are materials produced by epidermal cells that may modulate immunologic as well as inflammatory reactions. Such materials include thymic factors, prostaglandin E₂, leukotrienes, and interferon. The precise epidermal cell types producing these cytokines are not known. Keratinocytes secrete a factor, termed epidermal cell-derived thymocyte acti-

vating factor (ETAF), which is similar (if not identical) to interleukin 1.²⁹ Langerhans' cells also produce interleukin 1, which provides an important second signal in the activation of T cells. In addition, interleukin 1 has multiple other effects which include, but are not limited to, the induction of fibroblast proliferation, neutrophil chemotaxis, and the production of fever.²⁶

At present, there is no direct evidence linking these immunologic elements with the immunopathogenesis of urticaria, dermatitis herpetiformis, or atopic dermatitis. However, as indicated, the skin has the capability of responding immunologically in a variety of ways to various stimuli. As further developments in these areas unfold, the elements previously outlined may prove to be important in our understanding of and treatment approaches in these disorders.

There is, however, evidence that cutaneous mast cells participate in immunoinflammatory events associated with adverse reactions to foods. Mast cells are located within the dermis in greatest numbers near blood vessels, hair follicles, sebaceous glands, and sweat glands. Human skin contains approximately 7,000 mast cells/cu mm, which can undergo degranulation following challenge with a number of stimuli including allergens, compound 48/80,³⁰ opiates,³¹ substance P,³⁰ and histamine-releasing factors,³² among others. Mast cell heterogeneity, based on neutral protease composition, can also be demonstrated in the skin.³³ Cutaneous mast cell degranulation causes enhanced vascular permeability, produces itching or a sensation of burning, and induces leukocyte infiltration into the skin. These processes result in the development of both immediate wheal and flare reactions (resembling hives) that occur within minutes as well as late-phase inflammatory reactions (erythema and induration) that develop hours after challenge (see Fig 1).³⁴ The ability of various mast cell mediators, either alone or in concert, to contribute to tissue inflammation is now considered to be important to the pathogenesis of various cutaneous inflammatory processes such as urticaria and atopic dermatitis.

Urticaria and Angioedema

Clinical Features

Urticaria and angioedema can be defined temporally, clinically, histologically, and etiologically. It has been estimated that 10% to 20% of the population experiences at least one episode of acute urticaria or angioedema.³⁵ Chronic urticaria, defined as lesions that persist longer than 6 weeks, develops in only a small proportion of cases. In one report of children hospitalized for urticarial episodes, only 5% went on to develop chronic symptomatology.³⁶ Patients with chronic urticaria may have daily

symptoms or recurrent episodic eruptions, thus making the categorization of some patients difficult. In adults, the peak incidence of chronic urticaria is during the third and fourth decades, and it is more frequent in women.³⁷ Chronic urticaria is less common in children but its onset has been reported during the first month of life.³⁸ In contrast to chronic urticaria, the acute form tends to be more common in atopic individuals and more often has a definable cause. As mentioned previously, urticarial lesions may co-exist with angioedema. In one report evaluating 94 children less than 16 years of age, urticaria alone was observed in 85%, angioedema alone in 6%, and both in 9% of cases.³⁷

Urticarial lesions (hives) develop in the superficial layers of skin on any part of the body and usually have raised, erythematous, serpiginous edges and blanched centers. The pruritic wheals may vary in size from 1 to 2 mm to many centimeters (20 to 30) in diameter. Individual lesions do not leave residual macroscopic changes and seldom last longer than 24 to 48 hours; new lesions commonly arise as older ones fade. Angioedema, a similar process occurring in deeper subcutaneous layers, consists of colorless, well-demarcated edema that may or may not be pruritic. Most frequently, this edema occurs on the face (lips, tongue, and perioral and periorbital areas) and extremities.³⁹

Histopathology

Histologically, biopsy specimens of chronic urticarial lesions show edema and vascular dilation in the superficial part of the dermis.⁴⁰ A minimal cellular infiltrate is noted in the dermis, especially around small venules. The infiltrating cells consist predominantly of mononuclear cells and are accompanied occasionally by eosinophils and neutrophils. In comparison to the skin of normal individuals, lesional skin contains four times the number of cells and ten times the number of mast cells.⁴¹ Increased levels of histamine (present only in mast cells and basophils) both in lesional and nonlesional skin and in skin blister fluid (spontaneous release and in response to compound 48/80) have been described.⁴²⁻⁴⁴ Further, patients with chronic urticaria exhibit increased sensitivity to codeine skin testing⁴⁵; these findings indicate either that cutaneous mast cells in chronic urticaria patients are intrinsically more sensitive to degranulation or, alternatively, that the increased number of cutaneous mast cells noted by some investigators may trigger a larger end organ response. The mononuclear cell infiltrate is comprised predominantly of T-lymphocytes and approximately 70% of these are of the helper/inducer phenotype.⁴⁶ Sixty-five percent of these lymphocytes appear to be activated due to increased amounts of Ia surface antigen. As indicated in Table 1, lymphocytes may release factors that trigger mediator release. Taken together, these findings indicate a potential role either separately, or through cell-to-cell interactions, of mast cells and lymphocytes in the pathogenesis of urticaria.⁴⁷

Causes of Urticaria

In comparison to other factors, foods tend to be a relatively infrequent cause of urticarial episodes in children. IgE antibody-mediated urticaria can be seen following insect stings in children with hymenoptera sensitivity⁴⁸ or following penicillin therapy.⁴⁹ In addition to antigen interacting with IgE antibody to produce cutaneous mast cell mediator release, a number of other factors are known to trigger mast cell degranulation (see Table 1). An appreciation of the histopathology and the recognition that various physical or chemical agents may trigger mediator release is important when one considers the possible causes for urticaria and angioedema. Infections, including viral,⁵⁰⁻⁵³ bacterial,⁵⁴ and parasitic,^{55, 56} may cause urticaria through complement activation. Various drugs⁵⁷ such as radiocontrast material⁵⁸; aspirin⁵⁹⁻⁶¹ and other nonsteroidal antiinflammatory agents⁶²; narcotics^{63, 64}; antibiotics such as penicillin and its derivatives,⁶⁵ vancomycin,⁶⁶ and others⁶⁷; antineoplastics such as doxorubicin,⁶⁸ procarbazine,⁶⁹ and cyclophosphamide⁷⁰; anticonvulsants⁷¹; anesthetics⁷² and muscle relaxants⁷³; aspartame^{74, 75}; food dyes⁷⁶; and even steroids⁷⁷ have been reported to cause urticaria and/or angioedema. In some cases, IgE-antibody formation to these compounds can be demonstrated (penicillin); these agents may also trigger mediator release either through direct membrane effects or through complement activation. Physical causes such as pressure, sun exposure, cold, heat, exercise, water exposure, and vibration have been recently reviewed⁷⁸ and are known causes of urticaria. Consideration of cold-induced urticaria may be especially important in cases of chronic urticaria in children.^{37, 79} Physical urticarial lesions may result from mast cell activation,⁸⁰ some mediated through IgE antibody.⁸¹

Urticaria Following Contact With Food

Foods have been reported to cause urticaria in two ways: following contact or after oral ingestion. Contact urticaria is usually classified according to its presumed pathogenesis as immunologic (previous sensitization or passive transfer) and nonimmunologic contact urticaria (nonspecific release of vasoactive amines, negative passive transfer).⁸² Nonimmunologic contact urticaria is often caused by small-molecular-weight substances such as benzoic, cinnamic, and sorbic acids. The testing of these substances is often performed using a modified chamber test method for patch testing or an open test method; in both tests, the results are read 10 to 40 minutes after application of the test substance. In adults, neither stripping nor scratching the skin enhances the reaction.⁸³ Foods are rarely implicated in cases of nonimmunologic contact urticaria.⁸⁴ In one report, contact urticaria was observed in a kindergarten in 18 of 20 children following the intake and accidental perioral application of a mayonnaise salad cream.⁸⁴ Twenty-minute patch tests with the different components of the salad dressing were positive only for sorbic acid and benzoic acid.

In adults, several foods including potato, codfish, plaice, herring, lobster, and chicken have been reported to cause immunologic contact urticaria.⁸⁵⁻⁸⁷ In children, several reports^{83, 88} have implicated milk in this type of response. Salo and co-workers⁸³ performed open skin challenge tests with whole milk and its large- (>10,000 mw) and small-molecular-weight fractions on the intact skin of 51 children with atopic dermatitis and suspicion of milk allergy. Thirty-five of the 51 reacted within minutes with contact urticaria. The large-molecular-weight fraction (containing β -lactoglobulin, considered by some to be the most allergenic component) gave an urticarial reaction as often as whole milk, whereas the small-molecular-weight fraction gave only a few positive reactions. These results indicate that milk antigens can rapidly penetrate the skin and cause urticarial reactions in sensitized patients.

Urticaria Following Food Ingestion

Foods more commonly cause urticaria/angioedema following oral ingestion. Foods are more often a cause for acute rather than chronic urticaria. In acute urticaria, history alone can be very informative in establishing the culprit food. Certain foods tend to be more commonly involved in producing urticarial responses. These foods include milk, eggs, soy, nuts, peanuts and other legumes, fish, and seafood (including mollusks, crustacea, etc.). It is unknown why the composition of these foods tends to provoke IgE antibody responses in predisposed individuals. In general, urticaria/angioedema will occur within 1 hour following the ingestion of these foods. Reactions that begin at later time intervals are much less likely to be food related. In young children, milk and eggs tend to be more commonly implicated. Hill and co-workers⁸⁹ evaluated 100 young children (mean age, 16 months) with suspected milk allergy. Following open challenge, 31% developed urticaria within 45 minutes; the majority of these patients had either positive radioallergosorbent test (RAST) or skin tests to milk antigen indicating IgE sensitization. Kauppinen and co-workers⁹⁰ studied 163 children (ages 6 months to 16 years) with acute, recurrent, or chronic urticaria and found that foods (including milk, vegetables, and fish) were responsible for 15% of acute urticarial episodes. Food additives including azo-dyes, benzoate, and acetylsalicylic acid were incriminated in 8%.

Depending on the type of challenge procedure performed, foods or food additives have been variably implicated in the cause of chronic urticaria. Foods, including milk, vegetables, and fish, have been noted in one series⁹⁰ to be responsible for recurrent or chronic urticaria in 11% of cases. Harris and co-workers³⁸ found foods to be an uncommon cause (2%) of chronic urticaria in children. Due to the high number of false positives⁹¹ and to the unavailability of standardized extracts,⁹² immediate hypersensitivity skin testing or RAST testing with food antigens is not useful in chronic urticaria. Elimination diets, if performed cautiously to avoid inadequate nutritional intake, may be insightful. Double-blind food challenge can also be helpful.

Food additives have also been evaluated by a few investigators. Kemp and Schembi⁹³ placed 18 children with chronic urticaria on an elimination diet for 2 weeks and 7 of 18 (39%) had a marked remission in their symptoms. Challenge capsules containing aspirin, tartrazine, sodium benzoate, or yeast were then administered. Five of 14 challenged with aspirin (36%) reacted positively. Reactions to tartrazine, sodium benzoate, and yeast (7%) were no more numerous than to lactose placebo (9%). Supramaniam and Warner⁵⁴ studied 43 children with chronic urticaria who responded to an additive-free diet. Twenty-four of the children reacted to one or more additives following double-blind challenge. In the 18 who did not respond, all were placed on a normal diet and remained well. Kauppinen and co-workers⁹⁰ performed oral challenge tests and found that 15% of their recurrent and chronic urticaria patients were sensitive to acetylsalicylic acid, sodium benzoate, or tartrazine. These disparate findings indicate that careful analysis should be performed before incriminating food additives as a potential cause of chronic urticaria in children.

Treatment

Treatment of food-related acute and chronic forms of urticaria, as well as contact urticaria, involves establishing, either by history or by double-blind challenge, the culprit foods for each individual patient. The inherent problems with food allergen extracts and interpretation of immediate hypersensitivity skin test results have been reviewed.^{91, 92} Skin testing usually does not provide much useful additional information. Once a particular food (or food additive) has been convincingly established to produce a cause and effect relationship with the clinical symptomatology, avoidance of that food is usually curative.

Atopic Dermatitis

Clinical Features

In children, atopic dermatitis is the most common cause of "eczema," an inflammatory cutaneous eruption characterized by itching, erythema, edema, papules, vesicles, serous discharge, and crusting. In the first comprehensive description of atopic dermatitis, Besnier⁹⁵ emphasized the hereditary nature of this disorder, its chronically recurring course, and its association with hayfever and asthma. Wise and Sulzberger⁹⁶ further emphasized the relationship between atopic eczema, asthma, and "hayfever" (allergic rhinitis) by coining the term "atopic dermatitis," the term generally used today.

Atopic dermatitis affects between 1.9% and 4.3% of the pediatric population.^{97, 98} It has been estimated that approximately 1% of pediatric office visits in North America are related to atopic dermatitis.⁹⁹ Recent evi-

dence suggests that atopic dermatitis is not a unique affliction of industrialized society, as commonly believed. In a worldwide survey,¹⁰⁰ climatologic/geographic conditions, profession, and psychic stress appear to be the most consistent factors affecting the course of atopic dermatitis.

Atopic dermatitis frequently begins in early infancy. Sixty percent of individuals developing atopic dermatitis are affected by the first year of life, and 85% within the first 5 years.¹⁰¹ The rash generally begins as an erythematous, papulovesicular eruption that progresses (with scratching) to a scaly, lichenified rash over time.¹⁰² Distribution of the rash typically varies with age.¹⁰³ In the young infant (3–6 months to 2 years), flat, scaly lesions typically involve the cheeks and extensor surfaces of the arms and legs. Flexural surfaces, ankles, wrists, and neck are generally involved in the young child (2 to 12 years), with lichenification becoming a more prominent feature. In the teenage patient and young adult, extreme xerosis, prominent papulation, and lichenification in flexural surfaces and on the hands, feet, and face (especially about the eyes) are typical. Older patients tend to have symptom-free periods that may last for months, whereas the dermatitis is more constant in the younger years.

Unlike most dermatoses, atopic dermatitis has no primary skin lesion. Instead, it is identified by the presence of a constellation of symptoms, as recently outlined in the classification system proposed by Hanifin and Rajka (Table 5).¹⁰⁴ These criteria emphasize the extremely pruritic nature of the rash and its typical morphology, distribution, and tendency toward a chronic or relapsing course. Some features such as nipple eczema, upper lip cheilitis, and anterior subcapsular cataracts are infrequent, but relatively specific for the diagnosis of atopic dermatitis. Others, such as hyperlinearity of the palms, orbital darkening ("allergic shiners"), and Dennie-Morgan infraorbital folds are common but nonspecific symptoms.

Histopathology

Histologic changes in lesions of atopic dermatitis are not distinctive and may resemble those of contact dermatitis, id reactions, acute photoallergic reactions, and others.¹⁰⁵ Pathologic changes of the skin vary with the nature of the clinical lesion and can be subdivided into acute and chronic phases.¹⁰⁶ The acute lesion is characterized by slight psoriasiform hyperplasia of the epidermis with hyperkeratosis, spongiosis secondary to intracellular edema, and ballooning of the keratinocytes secondary to intracellular edema. Lymphocytes and occasional monocytes and macrophages infiltrate around venous plexes in the dermis. Mast cell numbers appear normal and there is no obvious increase in basophils or eosinophils.

Chronic lesions are characterized by moderate to marked hyperplasia of the epidermis with elongation of the rete ridges, prominent hyperkeratosis, and varying degrees of intercellular edema. Vesicle formation is not seen unless there is a superimposed acute exacerbation. Perivenular and intervascular areas are infiltrated with monocytes, macrophages, and lympho-

TABLE 5.
Diagnostic Features of Atopic Dermatitis*

Must have 3 or more major features:

Pruritus
Typical morphology and distribution
 Flexural lichenification or linearity in adults
 Facial and extensor involvement in infants and children
Chronic or chronically-relapsing course
Personal or family history of atopy (asthma, allergic
 rhinitis, or atopic dermatitis)

Must have 3 or more minor features:

Xerosis
Ichthyosis/palmar hyperlinearity/keratosis pilaris
Immediate (type I) skin test reactivity
Elevated serum IgE
Early age of onset
Tendency toward cutaneous infections (especially
 Staphylococcus aureus and herpes simplex)/impaired
 cell-mediated immunity
Tendency toward nonspecific hand or foot dermatitis
Nipple eczema
Cheilitis
Recurrent conjunctivitis
Dennie-Morgan infraorbital fold
Keratoconus
Anterior subcapsular cataracts
Orbital darkening
Facial pallor/facial erythema
Pityriasis alba
Itch when sweating
Intolerance to wool and lipid solvents
Perifollicular accentuation
Food hypersensitivity
Course influenced by environmental/emotional factors
White dermographism/delayed blanch

*From Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; 92(suppl):44-47. Used by permission.

cytes. Mast cell and Langerhans' cell numbers are significantly increased. Capillary number is often increased and capillary walls may be thickened. Demyelination and fibrosis of cutaneous nerves are seen at all levels of the dermis.

Immunohistochemical analyses¹⁰⁷ of the dermal infiltrate in atopic dermatitis lesions demonstrate that the mononuclear infiltrate consists predominantly of T cells bearing the CD3, CD4 surface antigens (T helper phenotype). T cells bearing the CD3, CD8 antigens (T suppressor/cytotoxic phenotype) are seen less frequently. Most of the cells in the infiltrate are highly positive for HLA class II antigens suggesting that they are "activated." B cells or plasma cells are rarely seen. Increased numbers of Langerhans' cells also are seen in chronic lesions as detected by the CD6 surface antigen. Langerhans' cells bear IgE molecules on their surface, a finding unique to patients with atopic dermatitis.²⁸ The infiltrate in the epidermis is reportedly similar to the dermis.

Immunopathology

Despite advances in our understanding of the immunopathology of atopic dermatitis, the etiology of this disorder remains unknown. Clearing of the eczematous rash in patients with Wiskott-Aldrich syndrome following successful reconstitution by bone marrow transplantation¹⁰⁸ and transfer of latent atopy in a child reconstituted with bone marrow from his atopic sibling¹⁰⁹ suggest that a bone marrow-derived cell (or factor) plays a central role in the immunopathology of atopic dermatitis.

Both humoral and cellular immune abnormalities have been described in patients with atopic dermatitis including elevated serum IgE concentrations, defective delayed-type skin responsiveness to various antigens, decreased lymphocyte response to mitogens, recall antigens, and alloantigens *in vitro*, and decreased phagocytic capacity and chemotaxis of neutrophils and monocytes.¹¹⁰ Interestingly, humoral and cellular abnormalities all vary directly with the clinical course of the skin lesions, raising the question of what is cause and what is effect.

Several factors suggest a central pathogenic role for an IgE-mediated mechanism(s) in atopic dermatitis.¹¹¹ Approximately 80% of children have elevated serum IgE concentrations and positive immediate skin tests to various dietary and environmental allergens; two-thirds have a positive family history of atopy and 50% to 80% of children develop allergic rhinitis and/or asthma. However, an IgE-mediated mechanism is frequently discounted because these findings are not uniform and because histologic findings of eczematous skin lesions suggest a classic cell-mediated (type IV) hypersensitivity reaction.¹¹²

The apparent discrepancy between the histologic findings and the strong clinical association of atopic dermatitis with IgE-mediated diseases has been addressed in the past several years. IgE-mediated responses often involve both an "immediate" and "late-phase" component (see Fig

1).^{113, 114} During the early phase of mast cell activation, chemotactic factors are generated that attract neutrophils and eosinophils to the area. Eosinophils and neutrophils infiltrate the area 4 to 8 hours after initial mast cell activation, followed by lymphocytes and monocytes at 1 to 2 days. Eosinophils may release a variety of potent inflammatory mediators: eosinophil major basic protein (MBP), a cytolytic protein known to damage skin epithelial cells and to promote further mast cell histamine release; eosinophil-derived neurotoxin (EDN), a powerful neurotoxin that may account for the demyelination of dermal nerve endings seen in atopic dermatitis skin; eosinophil cationic protein (ECP), another potent neurotoxin that inhibits lymphocyte proliferation *in vitro* and may play a role in the cutaneous anergy seen in patients with atopic dermatitis; and leukotriene C₄ (LTC₄) and platelet-activating factor (PAF), both of which possess proinflammatory activity.¹¹⁵ Although eosinophils are rarely seen in chronic atopic dermatitis lesions, immunofluorescent staining of skin biopsy specimens reveals large quantities of MBP as well as the typical mononuclear round cell infiltrate.¹¹⁶ MBP is not seen in uninvolved skin from the same patients or in lesions of patients with contact dermatitis.

Atopic Dermatitis Following Food Ingestion

Several early studies unequivocally demonstrated that ingested food allergens rapidly cross the gastrointestinal barrier, are transported to the periphery, and activate cutaneous mast cells.^{117, 118} Subsequently, a variety of immunologic mechanisms have been proposed to account for eczematous symptoms provoked by food allergens in patients with atopic dermatitis. However, only IgE-mediated hypersensitivity has been clearly implicated in controlled clinical trials.

In patients with IgE-mediated food hypersensitivity, ingestion of a food allergen results in an "immediate phase" during which time histamine¹¹⁹ and other preformed mediators are released. The cutaneous pruritus that is provoked results in scratching, subsequent excoriation, and eventually lichenification, as first proposed in the classic study of Engman et al.¹²⁰

The IgE-mediated "late phase" has also been implicated by controlled oral food challenges. Two food allergic patients, who experienced clearing of their eczema after maintaining appropriate food allergen restricted diets, underwent repeat double-blind placebo-controlled oral food challenge (DBPCFC).¹²¹ Both patients developed a pruritic morbilliform skin rash within 30 to 60 minutes. Skin biopsy specimens obtained from involved sites 4 and 14 hours later revealed an infiltration of eosinophils and deposition of MBP. This study demonstrated that ingested food allergens can activate mast cells and trigger both an immediate and late-phase response in the skin. The exact role of eosinophils and MBP in the pathogenesis of eczematous skin lesions remains to be established. However, its presence strongly implicates the pathogenic involvement of a late-phase IgE-mediated hypersensitivity response and provides an explanation for the typi-

cal mononuclear round cell infiltrate found in skin lesions of patients with atopic dermatitis.

As discussed previously, investigators have found that factors produced by mononuclear cells^{12, 13} and platelets¹⁴ can activate mast cells and basophils. These factors, termed "histamine-releasing factors," appear to activate the cells by binding to surface-bound IgE molecules.¹²² Similar factors are produced spontaneously *in vitro* by mononuclear cells from food sensitive patients with atopic dermatitis.¹²³ These factors may bind to cutaneous mast cells, or potentially other cells with surface-bound IgE (e.g., Langerhans' cells), and provoke the more chronic inflammation associated with eczematous lesions.

Several studies have suggested that adverse food reactions play a pathogenic role in some children with atopic dermatitis.¹²⁴⁻¹²⁷ Methods utilized to diagnose food hypersensitivity in these studies have varied. Consequently, there is disagreement concerning immunologic mechanisms involved. Using the DBPCFC to confirm the diagnosis of food hypersensitivity, 160 children referred for evaluation of severe eczema were evaluated for food allergies.¹²¹ All patients fulfilled the criteria of Hanifin and Rajka^{101, 104} for the diagnosis of atopic dermatitis. The median age for the group was 5.3 years, and in general, subjects were highly atopic. In addition to atopic dermatitis, approximately 50% had allergic rhinitis and asthma at the time of first evaluation, about 25% had allergic rhinitis, and 5% had asthma.

Prior to admission to a hospital research unit, patients were instructed to avoid any foods suspected of provoking allergic symptoms for 7 to 10 days. In addition, any medications that could interfere with interpretation of challenge studies (e.g., β -agonists, antihistamines) were discontinued. All patients underwent prick skin testing to a standard battery of food extracts prior to initiating DBPCFCs. Food challenges consisted of administering 8 to 10 gm of dehydrated food, or placebo, in capsules or camouflaged in a juice over a 1-hour period.² Over 500 DBPCFCs have been performed in the evaluation of these children. Diagnosis of food hypersensitivity was made by history in 25 instances because the patient provided a "convincing" account of a life-threatening reaction that required emergency room management. In each case, the patient had a strongly positive prick skin test to the food allergen in question.

Overall, 575 positive prick skin tests were elicited in the 160 children, nearly three times more than the 204 food hypersensitivities confirmed by blinded challenge or "convincing" history. (It should be noted that a skin test was only considered positive if it yielded a wheal at least 3 mm greater than the negative control.) All symptoms elicited by DBPCFC occurred within 2 hours of the challenge. Cutaneous reactions developed in about 80% of cases and consisted of a pruritic, erythematous, macular, or morbilliform rash. Urticarial lesions were rarely seen and generally consisted of only two or three lesions. Gastrointestinal symptoms were seen in 43% of the positive challenges, even though a history of gastrointestinal symptoms

was rarely elicited. Gastrointestinal symptoms included nausea, abdominal pain, vomiting, and/or diarrhea. Respiratory symptoms generally involved the upper respiratory tract and were seen in 28% of the positive DBPCFCs. Respiratory symptoms included nasal congestion, rhinorrhea, sneezing, stridor, and/or wheezing.

Of the 98 children found to have food hypersensitivity (60% of patients evaluated), five were infants who were being exclusively breast-fed. All five infants cleared their eczematous lesions when their mothers excluded eggs from their diet. The infants and their mothers were admitted to the hospital where the babies were challenged indirectly by feeding eggs to their mothers. All five infants developed an erythematous rash in the typical distribution of infantile atopic dermatitis. Food hypersensitivity reactions in breast-fed infants have been reviewed recently.¹²⁸

As shown in Table 6, six foods accounted for about 90% of the food hypersensitivities. Other foods eliciting reactions in two or more patients included chicken (4), potato (3), pork, beef, shrimp, and rice. Foods frequently claimed to exacerbate skin symptoms, such as chocolate, tomatoes, and citrus fruit, have never been confirmed by DBPCFC. Despite the large number of positive prick skin tests elicited in many children, 80% of subjects diagnosed with food hypersensitivity reacted to only one or two foods. Allergy to foods was highly specific; only one patient reacted to more than one food within a botanical family or animal species. Thus, the

TABLE 6.
Foods Eliciting
Hypersensitivity Reactions in
Children With Atopic
Dermatitis*

Food	No. of Patients	% of Reactions
Egg	78 (4)	38%
Peanut	48 (16)	24%
Milk	22	11%
Fish	15 (4)	7%
Soy	10	5%
Wheat	8	4%
Others	23 (1)	11%

*Diagnosis made by "convincing" history of a severe reaction following an isolated ingestion of a food is indicated by parentheses.

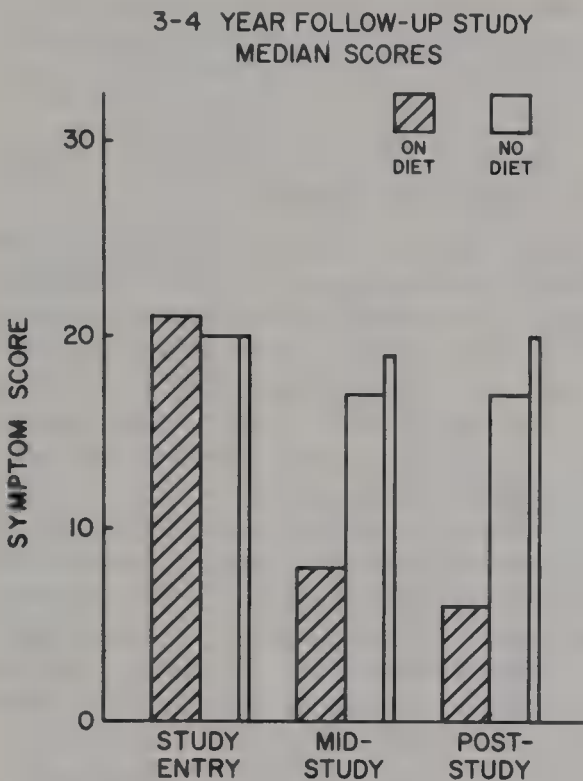
frequently recommended practice of avoiding all foods within a botanical family (e.g., all legumes, such as peanut, soy, green pea, green bean, pinto bean) is inappropriately restrictive and unnecessary.

Treatment

Treatment of food-related atopic dermatitis involves the accurate identification of the responsible food allergen(s). Negative prick skin tests (or RASTs) are useful for excluding potential food allergens⁹¹ but positive tests merely suggest potential sensitivities that must be confirmed by an oral challenge procedure. Brief trials (<3 weeks) of empirical elimination diets may be instituted to determine whether food allergy is contributing to a patient's symptoms. However, when improvement in the patient's symptoms is equivocal, or when more than one or two foods are implicated, blinded challenges must be undertaken to diagnose food hypersensitivity. Identifying food additives that may exacerbate eczematous skin symptoms also requires the use of brief elimination diets and blinded challenge studies. In the only controlled study of food additives in children with atopic dermatitis, no additive could be implicated in the pathogenesis of the disorder.¹²⁹ Once properly identified, the only treatment for food hypersensitivity is strict avoidance of the offending food. Acquiring the assistance of a knowledgeable dietitian and educating the patient and parent to read food labels has been found to be the most successful means of maintaining an effective elimination diet.¹³⁰ Other modalities proposed such as rotational diets, oral cromolyn, immunotherapy ("allergy shots"), and sublingual or subcutaneous neutralization have never been shown to be efficacious in well-controlled clinical trials.

In atopic dermatitis patients where food hypersensitivity plays a significant pathogenic role in their disease, symptoms improve readily once an appropriate elimination diet has been instituted. As shown in Figure 2, the clinical course of these patients is markedly improved compared with patients who are food sensitive but fail to comply with their diet or patients who are not food sensitive. Fortunately, food hypersensitivity is often "outgrown." It was found that approximately one-third of these food allergic individuals lost their clinical sensitivity (food challenge negative, prick skin test and/or RAST positive) after 1 year on the elimination diet.

The pediatrician always considers prophylaxis as the best form of therapy. Recent well-controlled studies support the role of exclusive breast feeding in preventing atopic disease. In one study of 135 infants of atopic parents, babies exclusively breast-fed for 6 months had a prevalence of atopic dermatitis of 14% at 1 year of age compared with a prevalence of 35% in a similar group breast-fed only 3 months.¹³¹ In addition, the number of solid foods added to the diet in the first 4 months of life has been shown to be directly related to the probability of developing food hypersensitivity.¹³²

**FIG 2.**

Clinical symptom scores in 34 patients with atopic dermatitis followed prospectively for 3 to 4 years. *Hatched bars* represent patients on elimination diets; *closed bars* represent patients on no special diet. *Narrow open bars* represent five patients who were food sensitive but were noncompliant with the diet. (From Sampson HA: The role of food allergy and mediator release in atopic dermatitis. *J Allergy Clin Immunol*, in press. Used by permission.)

Dermatitis Herpetiformis

Clinical Features

In 1884, Louis Duhring first described the clinical features of dermatitis herpetiformis, also known as Duhring's disease.¹³³ DH is felt to be caused by an abnormal immunologic and inflammatory response to dietary gluten. DH is a rare disorder in children, but clinically and immunologically resembles its adult counterpart.¹³⁴ In children, the average age of onset is about 7 years¹³⁵; in adults, the commonest age of onset is from the late second to the fourth decade.¹³⁶ DH is more common in whites than in blacks and seen only rarely in Japanese. The immunologically proven disease persists indefinitely, although with varying severity.

The primary lesion of DH is either an erythematous papule, an urticarial-like plaque, or most commonly, a vesicle. The papules often evolve into vesicles, some of which can form groupings to form herpes-like (herpetiform) lesions. The lesions are usually symmetrical and involve the scalp, shoulders, elbows, knees, and buttocks. Palmar and plantar exudative bullous lesions may occasionally be seen in children.¹³⁷ Due to the intense itching, lesions may become excoriated and crusting may be present. Although this process may lead to hyperpigmented and hypopigmented areas, scarring rarely occurs.¹³⁶ Because of its pleomorphic appearance,

DH may be confused clinically with erythema multiforme, bullous pemphigoid, herpes, scabies, papular urticaria, as well as other skin conditions. In one study, atopic dermatitis was noted in 20% of 45 Hungarian and Finnish children with DH.¹³⁸

Association With Gluten-Sensitive Enteropathy

Adult and childhood DH is frequently associated with gluten enteropathy that is clinically asymptomatic. A gluten-dependent enteropathy has been observed in 85% to 95% of cases.¹³⁶ The lesions are histologically similar to the intestinal lesions of celiac disease, but in DH the gastrointestinal clinical symptoms are mild and not seen in all patients.^{139, 140} Ermacora and co-workers evaluated 76 children with DH.¹⁴¹ No skin lesions were detected in children younger than 10 months of age and the most frequent age of onset was between 2 and 7 years. By history, 38% had had chronic relapsing diarrhea during the first 2 years of life, but only eight patients had established diagnoses of malabsorption before the onset of DH. At the time when the diagnosis of DH was made, the weights of 15% and the lengths of 13% were below the tenth percentile, respectively. D-xylose was abnormal in 32% of patients. Biopsy specimens of the small intestine showed affected jejunal mucosa in 85%, subtotal atrophy in 48%, and partial atrophy in 38%. Overall, biopsy changes in the small intestine were detected in more than 90% of cases. The lack of correlation between the high frequency of gastrointestinal histologic alterations and the relatively lower frequency of either concomitant biochemical or clinical parameters is noteworthy and currently not well understood.

Pathogenesis

The storage proteins present in wheat, rye, barley, and oats contain the protein species that are capable of causing DH.¹⁴² These proteins have been characterized most extensively in wheat wherein the toxicity has been shown to be due to the gliadin fraction, which can be separated into 30 to 50 individual components. Some fractions are symptomatically more toxic than others, with α -gliadin being the most toxic fraction both in vivo and in vitro.¹⁴² The variations in the toxicities of various gliadin fractions may explain the differences in the capacity of various wheats to produce symptoms as well as some of the regional differences in the occurrence of gluten-sensitive enteropathy.¹⁴² The amino acid composition of the active principle within gliadin is unknown.

The precise immunopathogenesis of DH has not been established. However, a number of interesting features of this disease have been described. The histologic features of the skin lesions are dissimilar from those found within the intestine. Cutaneous DH lesions contain granulocytic cellular infiltrates with local accumulation of edema whereas the gastrointestinal lesions contain a chronic lymphocytic cellular infiltration.¹⁴² In both adults

and children, granular, discontinuous IgA deposits are found within the epithelial membrane zone, most heavily concentrated in the dermal papillae.^{136, 141} In most instances, they occur in association with complement deposits and can be found in both lesional and nonlesional skin.¹⁴² It is hypothesized that the IgA bound in DH skin may represent either antibody specific for certain skin proteins or antigen-antibody complexes bound specifically to the skin. Attempts to demonstrate gluten proteins or their protein fragments in skin using immunofluorescent methods have not been successful.^{143, 144} Alternatively, the IgA may have antigenic specificity that cross-reacts with skin components. Indeed, in adults, Seah and co-workers¹⁴⁵ found a serum antibody in DH patients that bound to reticulin in the connective tissue of rat and human organs. Their studies suggested that the antireticulin antibodies bound in skin cross-reacted with gluten and trapped circulating gluten-antigluten immune complexes in dermal papillae. Both serum antireticulin and antigliadin antibodies in children with DH have been described, with levels tending to fluctuate with diet.¹⁴⁶ IgA antibodies with other specificities, including endomysium¹⁴⁷ and human jejunum,¹⁴⁸ have also been reported. In celiac disease, gluten-specific IgE antibodies are not found and skin testing does not produce wheal and flare reactions. These results indicate that, in gluten-sensitive enteropathy, IgE-mediated mast cell mediator release does not contribute to the pathogenesis.

Treatment

Treatment of DH involves dietary elimination or topical dapsone. Ermacora and co-workers¹⁴¹ treated children with DH with a gluten-free diet only and all cutaneous manifestations disappeared in 82% within 1 to 6 months. Some were rechallenged with a normal diet and cutaneous symptoms returned over a variable period of time (1 to 26 weeks). Dapsone also was effective but side effects of anemia, methemoglobinemia, and hemolysis (in a patient with G-6-PD deficiency) made diet the treatment of choice.

References

1. Bock SA, Lee W, Remigio LK, May CD: Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978; 62:327-334.
2. Sampson HA: Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1983; 71:473-480.
3. Metcalfe DD: Food hypersensitivity. *J Allergy Clin Immunol* 1984; 73:749-762.
4. Lemanske RF, Atkins FM, Metcalfe DD: Gastrointestinal mast cells in health and disease: Part I. *J Pediatr* 1983; 103:177-184.
5. Lemanske RF, Atkins FM, Metcalfe DD: Gastrointestinal mast cells in health and disease: Part II. *J Pediatr* 1983; 103:343-351.

6. Anderson JA, Bahna S, Buckley J, et al: *Adverse Reactions to Foods*, publication no. 84-2442. US Dept of Health and Human Services, Public Health Service, National Institutes of Health, 1984.
7. Walker WA, Isselbacher KJ: Uptake of macromolecules by the intestine: Possible role in clinical disorders. *Gastroenterology* 1980; 67:531-541.
8. Brandtzaeg P: The humoral immune systems of the human gastrointestinal tract. *Monogr Allergy* 1981; 17:195-200.
9. Brandtzaeg P, Baklien K: Immunohistochemical studies of the formation and epithelial transport of immunoglobulins in normal and diseased human intestinal mucosa. *Scand J Gastroenterol* 1976; 36:1-14.
10. Haneberg B, Aarskog D: Human faecal immunoglobulins in healthy infants and children and in some with diseases affecting the intestinal tract or the immune system. *Clin Exp Immunol* 1975; 22:210-215.
11. O'Donnell MD, Ackerman SJ, Gleich GJ, et al: Activation of basophil and mast cell histamine release by eosinophil granule major basic protein. *J Exp Med* 1983; 157:1981.
12. Thuesen DO, Speck LS, Lett-Brown MA, et al: Histamine-releasing activity (HRA): I. Production by mitogen or antigen-stimulated human mononuclear cells. *J Immunol* 1979; 123:626-631.
13. Kaplan AP, Haak-Frendscho M, Fauci A, et al: A histamine-releasing factor from activated human mononuclear cells. *J Immunol* 1985; 135:2027-2032.
14. Orchard MA, Kagey-Sobotka A, Proud D, et al: Basophil histamine release induced by a substance from stimulated human platelets. *J Immunol* 1986; 136:2240-2244.
15. Lindholm S: Mast cells in the wall of the alimentary canal. *Acta Pathol Microbiol Immunol Scand* 1959; 132(suppl):11-20.
16. Mikhail GR, Miller-Milinska A: Mast cell population in human skin. *J Invest Dermatol* 1964; 43:274-280.
17. Morris HT, Zamcheck N, Gottlieb L: The presence and distribution of mast cells in the human gastrointestinal tract at autopsy. *Gastroenterology* 1963; 44:448-455.
18. Sanderson IR, Slavin G, Walker-Smith JA: Density of mucosal mast cells in the lamina propria of the colon and terminal ileum of children. *J Clin Pathol* 1985; 38:771-773.
19. Blenestock J, Befus AD, Pearce F, et al: Mast cell heterogeneity: Derivation and function, with emphasis on the intestine. *J Allergy Clin Immunol* 1982; 70:407-412.
20. Befus AD, Dyck N, Goodacre R, et al: Mast cells from the human intestinal lamina propria: Isolation, histochemical subtypes, and functional characterization. *J Immunol* 1987; 138:2604-2610.
21. Irani AA, Craig SS, DeBlois G, et al: Deficiency of the tryptase-positive, chymase-negative mast cell type in gastrointestinal mucosa of patients with defective T lymphocyte function. *J Immunol* 1987; 138:4381-4386.
22. Fox CC, Dvorak AM, Peters SP, et al: Isolation and characterization of human intestinal mucosal mast cells. *J Immunol* 1985; 135:483-491.
23. Wasserman SI: Mediators of immediate hypersensitivity. *J Allergy Clin Immunol* 1983; 72:101-114.
24. Woodley DT: Importance of the dermal-epidermal junction and recent advances. *Dermatologica* 1987; 174:1-10.
25. Manabe M, Ogawa H: Ultrastructural demonstration of anionic sites in base-

- ment membrane zone by cationic probes. *J Invest Dermatol* 1985; 84:19–21.
26. Katz SI: The skin as an immunologic organ: A tribute to Marion B. Sulzberger. *J Am Acad Dermatol* 1985; 13:530–538.
27. Breathnach SM, Katz SI: Cell-mediated immunity in cutaneous disease. *Hum Pathol* 1986; 17:161–167.
28. Bruynzeel-Koomen C, van Wichen DF, Toonstra L, et al: The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch Dermatol Res* 1986; 278:199–205.
29. Luger TA, Stadler BN, Katz SI, et al: Epidermal cell (keratinocyte) derived thymocyte-activating factor (ETAF). *J Immunol* 1981; 127:1493–1498.
30. Benyon RC, Lowman MA, Church MK: Human skin mast cells: Their dispersion, purification, and secretory characterization. *J Immunol* 1987; 138:861–867.
31. Casale TB, Bowman S, Kaliner M: Induction of human cutaneous mast cell degranulation by opiates and endogenous opioid peptides: Evidence for opiate and nonopiate receptor participation. *J Allergy Clin Immunol* 1984; 73:775–781.
32. Warner JA, Pienkowski MM, Plaut M, et al: Identification of histamine releasing factor(s) in the late phase of cutaneous IgE-mediated reactions. *J Immunol* 1986; 136:2583–2587.
33. Irani AA, Schecter NM, Craig SS, et al: Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci USA* 1986; 83:4464–4468.
34. Lemanske R, Kaliner M: Mast cell-dependent late phase reactions. *Clin Immunol Rev* 1981–82; 1(4):547–580.
35. Twarog FJ: Urticaria in childhood: Pathogenesis and management. *Pediatr Clin North Am* 1983; 30:887–897.
36. Fineman S, Ghory JE. The hospitalized child with urticaria. *J Asthma Res* 1976; 14:27–35.
37. Champion RH, Roberts SOB, Carpenter RG, et al: Urticaria and angioedema: A review of 554 patients. *Br J Dermatol* 1969; 81:588.
38. Harris A, Twarog FJ, Geha RS: Chronic urticaria in childhood: Natural course and etiology. *Ann Allergy* 1983; 51:161–165.
39. Twarog FJ: Urticaria in childhood: Pathogenesis and management. *Pediatr Clin North Am* 1983; 30:887–898.
40. Mathews KP: Urticaria and angioedema. *J Allergy Clin Immunol* 1983; 72:1–14.
41. Natbony SF, Phillips ME, Elias JM, et al: Histologic studies of chronic idiopathic urticaria. *J Allergy Clin Immunol* 1983; 71:177–183.
42. Kaplan AP, Horakova Z, Katz S: Assessment of tissue fluid histamine levels in patients with urticaria. *J Allergy Clin Immunol* 1978; 61:350–355.
43. Phanuphak P, Schocket AL, Arroyave CM, et al: Skin histamine in chronic urticaria. *J Allergy Clin Immunol* 1980; 65:371–375.
44. Bedard PM, Brunet C, Pelletier G, et al: Increased compound 48/80 induced local histamine release from nonlesional skin of patients with chronic urticaria. *J Allergy Clin Immunol* 1986; 78:1121–1125.
45. Cohen RW, Rosenstreich DL: Discrimination between urticaria-prone and other allergic patients by intradermal skin testing with codeine. *J Allergy Clin Immunol* 1986; 77:802–806.

46. Mekori YA, Giorno RC, Anderson P, et al: Lymphocyte subpopulations in the skin of patients with chronic urticaria. *J Allergy Clin Immunol* 1983; 72:681-687.
47. Rosenstreich DL: Chronic urticaria, activated T cells, and mast cell releasability. *J Allergy Clin Immunol* 1986; 78:1099-1101.
48. Valentine MD: Insect venom allergy: Diagnosis and treatment. *J Allergy Clin Immunol* 1984; 73:299-304.
49. Sher TH: Penicillin hypersensitivity: A review, in Speck WT, Blumer JL (eds): *The Pediatric Clinics of North America*. Philadelphia, WB Saunders Co, 1983, pp 161-176.
50. Cherry JD, Jahn CL: Virologic studies of exanthems. *J Pediatr* 1966; 68:204-214.
51. Segool RA, Lejtenyl C, Taussig LM: Articular and cutaneous prodromal manifestations of viral hepatitis. *J Pediatr* 1975; 87:709-712.
52. Schuller DE: Acute urticaria in children: Causes and an aggressive diagnostic approach. *Postgrad Med* 1982; 72:179-185.
53. Lemanske RF, Bush RF: Cold-induced urticaria in infectious mononucleosis. *JAMA* 1982; 247:1604-1605.
54. Schuller DE, Elvey SM: Acute urticaria associated with streptococcal infection. *Pediatrics* 1980; 65:592-596.
55. Hamrick HJ, Moore GW: Giardiasis causing urticaria in a child. *Am J Dis Child* 1983; 137:761-763.
56. Cohen SG, Stapinski SM: Enteritis and urticaria associated with *Trichomonas hominis* infection. *Am Pract* 1954; 5:238-240.
57. Arndt KA, Jick H: Rates of cutaneous reactions to drugs: A report from the Boston collaborative drug surveillance program. *JAMA* 1976; 235:918-923.
58. Erffmeyer JE, Siegle RL, Leiberman P: Anaphylactoid reactions to radiocontrast material. *J Allergy Clin Immunol* 1985; 75:401-410.
59. Genton C, Frei PC, Pecoud A: Value of oral provocation tests to aspirin and food additives in the routine investigation of asthma and chronic urticaria. *J Allergy Clin Immunol* 1985; 76:40-45.
60. Botey J, Ibero M, Malet A, et al: Aspirin-induced recurrent urticaria and recurrent angioedema in non-atopic children. *Allergy* 1984; 39:265-267.
61. Settupane RA, Constantine HP, Settupane GA: Aspirin intolerance and recurrent urticaria in normal adults and children. *Allergy* 1980; 35:149-154.
62. Szczeklek A, Gryzlewski RJ, Czernowska-Myrek G: Clinical patterns of hypersensitivity to non-steroidal anti-inflammatory drugs and their pathogenesis. *J Allergy Clin Immunol* 1978; 60:276-280.
63. Casale TB, Bowman S, Kaliner M: Induction of human cutaneous mast cell degranulation by opiates and endogenous opioid peptides: Evidence for opiate and nonopiate receptor participation. *J Allergy Clin Immunol* 1984; 73:775-781.
64. Ebertz JM, Hermens JM, McMillan JC, et al: Functional differences between human cutaneous mast cells and basophils: A comparison of morphine-induced histamine release. *Agents Actions* 1986; 18:455-462.
65. Sullivan TJ: Allergic reactions to penicillin and other beta-lactam antibiotics, in Nelson JD, McCracken GH (eds): *Clinical Reviews in Pediatric Infectious Disease*. Philadelphia, BC Decker, 1985, pp 89-96.
66. McHenry MC, Gavan TL: Vancomycin, in Speck WT and Blumer JL (eds): *The Pediatric Clinics of North America*. Philadelphia, WB Saunders Co, 1983, pp 31-48.

67. Sullivan TJ: Allergic reactions to antimicrobial agents: A review of reactions to drugs not in the beta lactam antibiotic class. *J Allergy Clin Immunol* 1984; 74:594-599.
68. Souhami L, Feld R: Urticaria following intravenous doxorubicin administration. *JAMA* 1978; 240:1624-1626.
69. Glovsky MM, Braunwald J, Opelz G, et al: Hypersensitivity to procarbazine associated with angioedema, urticaria, and low serum complement activity. *J Allergy Clin Immunol* 1976; 57:134-140.
70. Kim HC, Kesarwala HH, Colvin M, et al: Hypersensitivity reaction to a metabolite of cyclophosphamide. *J Allergy Clin Immunol* 1985; 76:591-594.
71. Knutsen AP, Anderson J, Satayaviboon S, et al: Immunologic aspects of phenobarbital hypersensitivity. *J Allergy Clin Immunol* 1984; 105:558-563.
72. Lilly JK, Hoy RH: Thiopental anaphylaxis and reagin involvement. *Anesthesiology* 1980; 53:335-337.
73. Vervloet D, Nizankowska E, Arnaud A, et al: Adverse reactions to suxamethonium and other muscle relaxants under general anesthesia. *J Allergy Clin Immunol* 1983; 71:552-558.
74. Kulczycki A: Aspartame-induced urticaria. *Ann Intern Med* 1986; 104:207-208.
75. Szucs EF, Barrett KE, Metcalfe DD: The effects of aspartame on mast cells and basophils. *Food Chem Toxicol* 1986; 24:171-174.
76. Lockey SD: Hypersensitivity to tartrazine (FD&C yellow no. 5) and other dyes and additives present in foods and pharmaceutical products. *Ann Allergy* 1977; 38:206-210.
77. Mendelson LM, Meltzer EO, Hamburger RN: Anaphylaxis-like reactions to corticosteroid therapy. *J Allergy Clin Immunol* 1974; 54:125-131.
78. Jorizzo JL, Smith EB: The physical urticarias: An update and review. *Arch Dermatol* 1982; 118:194-201.
79. Ting S: Cold-induced urticaria in infancy. *Pediatrics* 1984; 73:105-106.
80. Soter NA, Wasserman SI: Physical urticaria/angioedema: An experimental model of mast cell activation in humans. *J Allergy Clin Immunol* 1980; 66:358-365.
81. Houser DD, Arbesman CE, Ito K, et al: Cold urticaria: Immunologic studies. *Am J Med* 1970; 49:23-33.
82. Maibach HI, Johnson HL: Contact urticaria syndrome. *Arch Dermatol* 1975; 111:726-730.
83. Salo OP, Makinen-Kiljunen S, Juntunen K: Milk causes a rapid urticarial reaction on the skin of children with atopic dermatitis and milk allergy. *Acta Derm Venereol (Stockholm)* 1986; 66:438-442.
84. Clemmensen O, Hjorth N: Perioral contact urticaria from sorbic acid and benzoic acid in a salad dressing. *Contact Dermatitis* 1982; 8:1-6.
85. Hannuksela M, Lahti A: Immediate reactions to fruits and vegetables. *Contact Dermatitis* 1977; 3:79-84.
86. Hjorth N, Roed-Petersen J: Occupational protein contact dermatitis in food handlers. *Contact Dermatitis* 1976; 2:28-42.
87. Maibach HI: Immediate hypersensitivity in hand dermatitis. *Arch Dermatol* 1976; 112:1289-1291.
88. Edwards EK: Contact urticaria to cow's milk. *Cutis* 1981; 28:450-451.
89. Hill DJ, Firer MA, Shelton MJ, et al: Manifestations of milk allergy in infancy: Clinical and immunologic findings. *J Pediatr* 1986; 109:270-276.
90. Kauppinen K, Juntunen K, Lanki H: Urticaria in children: Retrospective evaluation and follow-up. *Allergy* 1984; 39:469-472.

91. Sampson HA, Albergo R: Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984; 74:26–33.
92. Lemanske RF, Taylor SL: Standardized extracts, foods. *Clin Rev Allergy* 1987; 5:23–36.
93. Kemp AS, Schembri G: An elimination diet for chronic urticaria of childhood. *Med J Aust* 1985; 143:234–235.
94. Supramaniam G, Warner JO: Artificial food additive intolerance in patients with angio-oedema and urticaria. *Lancet* 1986; 2(8512):907–990.
95. Hanifin JM: Atopic dermatitis, in Safari B, Good RA (eds): *Comprehensive Immunology*, no. 7. New York, Plenum Medical Book Co, 1981, p 301.
96. Wise F, Sulzberger MB: *Yearbook of Dermatology and Syphilology*. Chicago, Year Book Medical Publishers, Inc, 1933, p 59.
97. Johnson ML, Roberts J: Prevalence of dermatologic disease among persons 1–74 years of age: United States, PHS no. 79–1660. Dept of Health, Education, and Welfare, 1979.
98. Halpern SR, Sellars WA, Johnson RB, et al: Development of childhood allergy in infants fed breast, soy, or cow milk. *J Allergy Clin Immunol* 1973; 51:139–151.
99. Krafchik BR: Atopic dermatitis. *Pediatr Clin North Am* 1983; 30:669–685.
100. Rajka G: Atopic dermatitis: Correlation of environmental factors with frequency. *Int J Dermatol* 1986; 25:301–304.
101. Rook A (ed): *Major Problems in Dermatology: Atopic Dermatitis*, vol 3. Philadelphia, WB Saunders Co, 1975.
102. Blaylock WK: Atopic dermatitis: Diagnosis and pathobiology. *J Allergy Clin Immunol* 1976; 57:62–69.
103. Hill LW, Sulzberger MG: Evaluation of atopic dermatitis. *Arch Dermatol Syph* 1935; 32:62–69.
104. Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Dermatol Venereol* 1980; 92(suppl):44–47.
105. Ackerman AB: Histopathologic differentiation of eczematous dermatitides from psoriasis and seborrheic dermatitis. *Cutis* 1977; 20:619–623.
106. Tong AKF, Mihm MC: The pathology of atopic dermatitis. *Clin Rev Allergy* 1986; 4:27–42.
107. Leung DYM, Bhan AK, Schneeberg EE, et al: Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* 1983; 71:47–56.
108. Saurat J-H: Eczema in primary immune-deficiencies. *Acta Derm Venerol (Stockh)* 1985; 114:125–128.
109. Saarinen UM: Transfer of latent atopy by bone marrow transplantation: A case report. *J Allergy Clin Immunol* 1984; 74:196–200.
110. Leung DYM, Geha RS: Immunoregulatory abnormalities in atopic dermatitis. *Clin Rev Allergy* 1986; 4:67–86.
111. Sampson HA: The role of “allergy” in atopic dermatitis. *Clin Rev Allergy* 1986; 4:125–138.
112. Mihm MC, Soter NA, Dvorak HF, et al: The structure of normal skin and the morphology of atopic eczema. *J Invest Dermatol* 1976; 67:305–312.
113. Dolovich J, Hargreave FE, Chalmers R, et al: Late cutaneous allergic responses in isolated IgE-dependent reactions. *J Allergy Clin Immunol* 1973; 52:38–46.

114. Solley GO, Gleich GJ, Jordan RE, et al: The late phase of the immediate wheal and flare skin reaction: Its dependence upon IgE antibodies. *J Clin Invest* 1976; 58:408–420.
115. Gleich GJ, Leiferman KM: Eosinophils and hypersensitivity disease, in Reed CE (ed): *Proceedings of the XII International Congress of Allergology and Clinical Immunology*. St Louis, CV Mosby Co, 1986, pp 124–130.
116. Leiferman KM, Ackerman SJ, Sampson HA, et al: Dermal deposition of eosinophil granule major basic protein in atopic dermatitis: Comparison with onchocerciasis. *N Engl J Med* 1985; 313:282–285.
117. Brunner M, Walzer M: Absorption of undigested proteins in human beings: The absorption of unaltered fish protein in adults. *Arch Intern Med* 1928; 42:173–179.
118. Wilson SJ, Walzer M: Absorption of undigested proteins in human beings IV: Absorption of unaltered egg protein in infants. *Am J Dis Child* 1935; 50:49–54.
119. Sampson HA, Jolie PA: Increased plasma histamine concentrations after food challenges in children with atopic dermatitis. *N Engl J Med* 1984; 311:372–376.
120. Engman WF, Weiss RS, Engman MF: Eczema and environment. *Med Clin North Am* 1936; 10:651–663.
121. Sampson HA: Immunologically mediated reactions to foods: IgE in eczema. *Ann Allergy* 1987; 59:71–76.
122. MacDonald SM, Lichtenstein LM, Proud D, et al: Studies of IgE-dependent histamine releasing factors: Heterogeneity of IgE. *J Immunol* 1987; 139:506–512.
123. Sampson HA, Broadbent K: “Spontaneous” basophil histamine release and histamine releasing factor in patients with atopic dermatitis and food hypersensitivity. *J Allergy Clin Immunol* 1987; 79:241.
124. Goldman AS, Anderson DW, Sellers WA, et al: Milk allergy: 1. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963; 32:425–443.
125. Hammar H: Provocation with cow’s milk and cereals in atopic dermatitis. *Acta Derm Venereol (Stockh)* 1977; 57:159–163.
126. Atherton DJ, Sewell M, Soothill JF, et al: A double-blind controlled crossover trial of an antigen avoidance diet in atopic eczema. *Lancet* 1978; 1:401–403.
127. Sampson HA, McCaskill CM: Food hypersensitivity in atopic dermatitis: Evaluation of 113 patients. *J Pediatr* 1985; 107:669–675.
128. Gerrard JW: Allergies in breast-fed infants. *Clin Allergy* 1984; 2:143–149.
129. Hannuksela M, Lahti A: Peroral challenge tests with food additives in urticaria and atopic dermatitis. *Int J Dermatol* 1986; 25:178–180.
130. Leinhas JL, McCaskill CM, Sampson HA: Food allergy challenges: Guidelines and implications. *J Am Diet Assoc* 1987; 87:604–608.
131. Saarinen UM: Prophylaxis for atopic disease: Role of infant feeding. *Clin Rev Allergy* 1984; 2:151–167.
132. Fergusson DM, Horwood LJ, Beutrais AL, et al: Eczema and infant diet. *Clin Allergy* 1981; 11:325–331.
133. Duhring L: Dermatitis herpetiformis. *JAMA* 1884; 3:225–228.
134. Gellis SE: Bullous diseases of childhood. *Dermatol Clin* 1986; 4:89–98.
135. Reunala T, Kosnai I, Karpati S: Dermatitis herpetiformis: Jejunal findings and skin response to gluten-free diet. *Arch Dis Child* 1984; 59:517–522.

136. Katz SI, Hall RP, Lawley TJ, et al: Dermatitis herpetiformis: The skin and the gut. *Ann Intern Med* 1980; 93:857-874.
137. Karpai S, Torok E, Kosnai I: Discrete palmar and plantar symptoms in children with dermatitis herpetiformis Duhring. *Cutis* 1986; 37:184-187.
138. Karpai S, Kosnai I, Verkasalo M, et al: HLA antigens, jejunal morphology, and associated diseases in children with dermatitis herpetiformis. *Acta Paediatr Scand* 1986; 75:297-301.
139. Brown JR, Parker F, Weinstein WM, et al: The small intestinal mucosa in dermatitis herpetiformis: Severity and distribution of small intestinal lesions and associated malabsorption. *Gastroenterology* 1971; 60:355-361.
140. Scott BB, Young S, Raja SM, et al: Coeliac disease and dermatitis herpetiformis: Further studies of their relationship. *Gut* 1976; 17:759-762.
141. Ermacora E, Prampolini L, Tribbia G, et al: Long-term follow-up of dermatitis herpetiformis in children. *J Am Acad Dermatol* 1986; 15:24-30.

Gastrointestinal Syndromes Associated With Food Sensitivity

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Immunologic reactions to dietary antigens (food sensitivity) are thought to play a role in a wide variety of food-related clinical syndromes in the pediatric age group.¹ In contrast to recent reviews,²⁻⁵ it is the purpose of this report to present the aspects of food sensitivity as they relate to specific areas of the digestive tract. Various gastrointestinal diseases in which food sensitivity is believed to play a role are discussed with respect to pathogenesis, evaluation, and therapy. In addition we briefly review the developmental aspects of the mucosal immune response that predispose the infant and child to food sensitivity-related gastrointestinal disease.

Developmental Aspects

Oral Tolerance

Throughout life, the human gastrointestinal tract is exposed to an unlimited number of dietary antigens. In spite of this continual attack on the mucosal defense mechanisms, adverse responses to nonself antigens of dietary origin are extremely infrequent, while effective immunity to infectious agents is maintained in the normal host. The homeostatic mechanism that allows us to maintain this state of "gastrointestinal unresponsiveness" is known as

oral tolerance. While a thorough explanation of our current understanding of the mechanisms of oral tolerance is beyond the scope of this discussion, several aspects deserve mention with regard to alterations that may be present in the human infant.

Much of our knowledge of the response to fed antigens comes from studies in experimental animals. It has been known for some time that the feeding of a single dose of ovalbumin to mice can effectively suppress systemic immune responses on reexposure to the same antigen. This suppression can be prolonged, may occur after feeding a range of doses of ovalbumin, and may abrogate systemic humoral (immunoglobulins [Ig] G, M, E) and cell-mediated (delayed-type hypersensitivity) immune responses.⁶ The mechanism for this suppression seems to involve the induction of antigen-specific suppressor T cells.^{7, 8} The specific events occurring at the mucosal or systemic level that lead to the induction of these antigen-specific suppressor T cells are not yet elucidated.

Alterations in the normal sequence of events resulting in oral tolerance have been detected in neonatal (as compared with adult) mice. The exposure to ovalbumin during the first week of life was associated with priming, rather than suppression, of humoral and cellular systemic immune responses on subsequent reexposure to the same antigen.^{9, 10} In addition, at the time of weaning, exposure to ovalbumin was transiently associated with priming and not tolerance. The effect of weaning was independent of the age of the animal at the time of weaning and ovalbumin exposure. Thus, both age at initial exposure to a fed protein antigen and the timing of exposure to new antigens relative to weaning may play a role in subsequent immune responses to the same antigen.

Two other aspects of the process of tolerance induction deserve mention because of their relevance to clinical food hypersensitivity in the infant. The first observation is that the process of exposure and induction of oral tolerance to a fed protein antigen may prevent the development of oral tolerance to a second antigen fed during the same time period.¹¹ Thus, it is conceivable that the simultaneous exposure to several new antigens may lead to the induction of tolerance to some and sensitivity to others.

The development of oral tolerance may also be affected by the nature of the antigen when presented to the immune system. Processing of fed protein antigens by either the intestinal mucosal absorptive cell or from partial degradation by proteolytic enzymes may lead to altered antigens that are more effective at inducing oral tolerance.^{12, 13} Factors that alter intestinal proteolysis or antigen presentation by the intestinal mucosa may ultimately affect the state of responsiveness of the organism on reexposure to the same antigen.

Intestinal Permeability

While the normal response to antigens that are introduced orally is the induction of a state of immune unresponsiveness, antigens that bypass the

normal mucosal immune barrier may trigger an immune reaction. Alterations of mucosal barrier function may allow penetration of a variety of noxious agents, including unprocessed protein antigens, which may ultimately result in immunologically mediated gastrointestinal disease.

Mucosal barrier function is the result of both nonimmunologic and immunologic defense mechanisms (Table 1) that each, individually, help to limit uptake of intact protein antigen. Luminal factors, such as gastric acid and intestinal enzymes, assist with hydrolysis and proteolysis of fed protein reducing the burden of intact protein presented to the mucosal absorptive surface.^{14, 15} Peristalsis expedites the clearance of antigens and antigen-antibody complexes that may adhere to the luminal surface.¹⁶ Intestinal mucous acts as a physical barrier to prevent penetration of antigen. In addition, mucous glycoproteins may impair attachment of luminal substances to cell surface receptors.¹⁷ Alterations of microvillus membrane composition may affect surface binding and diffusion of luminal molecules.¹⁸

Immunologic factors, such as mucosal IgA, are postulated to play a role in antigen exclusion by complexing to enteric antigens and preventing their transport across the intestinal epithelium.¹⁹ Antigen-antibody complexes that form as a result of this interaction have also been shown to stimulate goblet cell mucous release²⁰ and to enhance proteolysis of complexed antigens.¹⁵ Immune complexes that escape the mucosal barrier may be more readily cleared by the reticuloendothelial system than free antigen.²¹

The results of several experimental studies suggest that mucosal barrier function may be impaired in the newborn, leading to enhanced macromolecular uptake.²² Alterations in the composition of the microvillous membranes in newborns have been detected^{18, 23} and these alterations may facilitate attachment and transport of antigens in the newborn animal.²⁴ IgA concentrations in serum, saliva, and stool of newborns are re-

TABLE 1.
Components of the Mucosal Barrier

Nonimmunologic
Gastric acid
Proteolytic enzymes
Peristalsis
Intestinal mucous
Microvillus membrane
Immunologic
Mucosal IgA

duced compared with adults.^{25, 26} Immunofluorescent studies have demonstrated reduced numbers of IgA-containing cells in the intestinal mucosa of infants.²⁷ These observations, reflecting the enhanced permeability of the mucosal barrier in the newborn and altered mucosal immune responses to absorbed antigens, may help to explain why infants are predisposed to immune-mediated gastrointestinal disease.

Clinical Manifestations

Diagnostic Approach

The diagnostic approach to patients with suspected food sensitivity has been the subject of controversy over the past several years.

The diagnostic approach to a patient with possible food intolerance is based on history, physical exam, and diagnostic testing. Patients with suspected food sensitivity may give a history compatible with an adverse reaction to a specific dietary antigen; however, at the time of evaluation, there may be a paucity of physical findings. In the pediatric population, several studies have demonstrated that the history is unreliable in predicting who will be shown eventually to have documented food sensitivity.^{28, 29} Thus, diagnostic testing plays a critical role in establishing a definitive diagnosis of food sensitivity.

Evaluations of food sensitivity can be divided into two major groups: (1) serologic studies and skin tests and (2) oral challenge. In the first group, measurements of food-specific IgE,^{30, 31} circulating histamine levels,^{32, 33} leukocyte histamine release,^{34, 35} and skin testing^{36, 37} are directed at identifying mechanisms involving the IgE-mast cell axis (type I hypersensitivity). Similarly, assays to detect immune complexes (type III hypersensitivity),³⁸ complement abnormalities,^{39, 40} alterations in cell-mediated immunity (type IV hypersensitivity),⁴¹ and even explants of intestine placed in organ culture⁴² have been examined. None of these studies, however, achieve the diagnostic accuracy of oral challenge as originally described by Goldman et al.²⁹ In this landmark study, the diagnosis of milk allergy was accepted only when the following criteria were met:

- (1) Symptoms subsided following milk elimination; (2) Symptoms occurred within 48 hours following a trial feeding of milk; (3) Three such challenges were positive and similar as to onset, duration, and clinical features; (4) Symptoms subsided following each challenge reaction.²⁹

This approach was demonstrated to be more accurate than skin testing⁴³ and serologic studies⁴⁴ by the same and other authors.^{28, 45}

Although oral challenge has withstood the test of time, modifications of the original technique have been suggested based on the severity of the illness, cooperation of the patient, and therapeutic implications. The sever-

ity of the gastrointestinal reaction or associated systemic reaction (e.g., anaphylaxis) to a particular dietary antigen may lead to a reluctance on the part of the patient, parent, or physician to complete three challenges. For these severe reactions, double-blinded, in-hospital exposure to minute doses of the suspected antigen with close observation²⁸ may obviate the need for repeated oral challenge.

For less severe reactions the need to establish a definitive diagnosis based on blinded or repeated challenge needs to be evaluated with respect to implications for future dietary and medical management. For example, while several nutritionally adequate alternatives exist for the infant-fed cow's milk formula, nutritional consequences such as failure-to-thrive or kwashiorkor may result from misguided attempts to seek a very restrictive diet that leads to a decrease in symptoms.^{46, 47} Food sensitivity has also been described as the chief complaint of several children ultimately shown to have Munchausen's syndrome by proxy.⁴⁸ The objective demonstration of a lack of a clinical response to dietary antigens in these children may be an important first step to avoiding subsequent unwarranted diagnostic and therapeutic interventions.

While Goldman's criteria identify the presence of an adverse reaction to an ingested substance, they are not specific for immunologically mediated or hypersensitivity-type reactions. For example, the child with secondary lactase deficiency may have a stereotypic response to milk ingestion that may be related to lactose malabsorption and not to any immunologically mediated reaction within the gastrointestinal tract. Thus, it is important to also evaluate the possibility of associated carbohydrate malabsorption as the basis for the patient's symptoms.

Clinical Gastrointestinal Allergic Syndromes

Oral Lesions

A variety of interesting and unusual symptoms and signs have been reported as oropharyngeal manifestations of food sensitivity (Table 2). Some of these clinical syndromes seem to occur in association with atopic disease. Anderson et al.⁴⁹ reported that 6.2% of patients with pollen allergy complained of oral pruritus after ingestion of dietary antigens. Other, less frequently reported symptoms included swollen lips, pharyngeal pruritus, hoarseness, and aphthous lesions. Several patients with concomitant persistent aphthous ulceration and jejunal mucosal abnormalities have been described⁵⁰ and, in some of these patients, both the jejunal mucosal abnormalities and the aphthous lesions resolved on a gluten-free diet. A favorable response to gluten withdrawal has also been demonstrated in patients with aphthous ulceration and normal small bowel morphology.⁵¹

An interesting syndrome of chronic mucosal fissuring of the mouth has

TABLE 2.
Clinical Gastrointestinal Allergic Syndromes

Oral lesions
Oral pruritus
Aphthous stomatitis
Chronic mucosal fissuring
Esophagus and stomach
Vomiting
Eosinophilic gastroenteritis
Mucosal form: Anemia and hypoalbuminemia
Muscular form: Obstruction
Serosal form: Ascites
Small intestine
Milk- and soy-induced disease
Chronic blood loss
Protein-losing enteropathy
Chronic diarrhea
Gluten-sensitive enteropathy
Colon
Colitis

been noted in patients with recurrent episodes of oral angioedema and atopic symptoms. After repeated attacks of lip, tongue, and mouth swelling following the ingestion of specific foods, marked furrowing of the mucosa occurred.⁵² These changes have also been described in association with eosinophilic gastroenteropathy in which a biopsy specimen of the oral mucosa also revealed eosinophilic infiltration.⁵³

The pathogenesis of these varied examples of oral pathology, which may be related to food hypersensitivity, is poorly understood. A variety of environmental and physiologic factors have been implicated in the pathogenesis of aphthous ulceration. Studies examining deficient salivary IgA or increases in serum antibodies to food proteins in patients with aphthous ulceration have detected no consistent abnormalities.⁵⁴⁻⁵⁷

It is clear that oral pathology may be the presenting manifestation of food sensitivity and may herald the presence of pathology elsewhere in the gastrointestinal tract. Nevertheless, food sensitivity is believed to play a role in only a small percentage of oral diseases and should be documented by elimination and rechallenge with the suspected antigen. In these patients, the institution of restrictive diets should be reserved for those who have had a documented association by blinded oral challenge and should be tempered by the severity of the patient's symptoms.

Esophagus and Stomach

Food sensitivity is often divided into immediate- and delayed-type reactions for the purpose of classification. This distinction is critical for the evaluation of reactions involving the upper gastrointestinal tract (stomach and small intestine) where both immediate and delayed reactions may occur in the same patient.

After the mouth and oropharynx, the esophagus and the stomach are exposed to intact antigen immediately after ingestion. Thus, it is not surprising that prospective studies examining the response to oral challenge in infants and older children with suspected food sensitivity have demonstrated that vomiting is a common reaction to an offending antigen.^{28, 29} Emesis generally occurs as an immediate (less than 4 hours) reaction to oral challenge with a specific antigen. Because there frequently is a simultaneous occurrence of urticaria and/or wheezing,²⁸ it is believed that a poorly understood IgE-mast cell-dependent reaction is responsible for the initiation of the vomiting episode.⁵ The mechanism by which mast cell-derived mediators may act locally or centrally to induce emesis remains to be elucidated.

Eosinophilic gastroenteritis is an idiopathic condition in which many areas of the gastrointestinal tract, most commonly the stomach, are inflamed and diffusely infiltrated with eosinophils. The inflammatory lesion can be classified into three major clinical/pathologic entities involving (1) the mucosa, which leads to malabsorption and gastrointestinal loss of protein and blood, (2) the muscularis, which causes thickening of the bowel wall with symptoms and signs of obstruction, and (3) the serosa, which results in eosinophilic ascites.⁵⁸⁻⁶⁰ The clinical presentation depends on the anatomic site of gastrointestinal involvement as well as the affected layer of the bowel wall. Thus, children with mucosal involvement of the stomach and small intestine have edema and anemia due to intestinal loss of blood and protein. Diarrhea and weight loss may be present depending on the degree of associated malabsorption. Involvement of the muscularis of the gastrointestinal tract leads to obstruction at the level of the bowel at which this occurs. Serosal disease produces ascites with a predominance of eosinophils in the cellular exudate. With each of these clinical presentations, the gastric antrum seems to be the most common site of injury. Involvement of the esophagus, small bowel, colon, and other organ systems has been described.⁶¹⁻⁶⁴

The relationship of this eosinophilic inflammatory process to food hypersensitivity has been the subject of controversy. Eosinophilic gastroenteritis is often accompanied by symptoms of atopic diseases such as urticaria, eczema, rhinitis, or asthma. After ingestion of particular foods, patients with the "allergic" form of the disease may experience nausea, vomiting, abdominal pain, or diarrhea as well as an increase in their atopic symptoms. These responses may be both immediate and delayed. Increases in total serum or tissue IgE, an increase in serum IgE in response

to food challenge, positive radioallergosorbent tests (RASTs) to multiple foods, and positive skin tests to food extracts have been demonstrated in this form of the disease.^{65, 66} However, a "nonallergic" form of the disease also occurs in which the patients have no symptoms of atopic disease and show no correlation of their gastrointestinal symptoms to blinded food challenge. The nonallergic variety is associated with normal serum and tissue IgE levels and negative skin tests to food antigens.⁵⁹ These clinical observations suggest that both IgE-dependent and IgE-independent mechanisms may lead to eosinophilic infiltration of the gastrointestinal tract.

The diagnosis of eosinophilic gastroenteritis is often suggested by finding peripheral eosinophilia, which may be severe (absolute eosinophil count greater than $1,500/\text{mm}^3$), in a patient with compatible symptoms and signs. Additional supportive laboratory findings may include iron-deficiency anemia, hypoalbuminemia, or an elevated level of IgE. Skin tests or RASTs may be helpful in determining potential offending antigens. Radiologic studies may demonstrate nodularity or thickening of the gastric antrum or thickening of small bowel folds.⁶⁷ Mucosal biopsy of the affected portion of the gastrointestinal tract is necessary for demonstrating the pathologic lesion. The eosinophilic inflammatory process may be patchy in distribution, especially in the small intestine. The gastric antrum, however, is the site that most consistently demonstrates the pathologic lesion when multiple sites are biopsied.⁶¹ For this reason, antral biopsy is most helpful whenever symptomatology is related to upper gastrointestinal involvement. Blinded challenge may confirm the relationship to food sensitivity and may help determine appropriate dietary modifications as part of the therapeutic plan.

The response to elimination diets in both the allergic and nonallergic forms of the disease has been inconsistent. In general, infants are more likely to have a favorable response to the withdrawal of milk and/or soy from their diet than older children.⁶⁸ Infants who respond to dietary therapy are also more likely to have a self-limited disease process that improves with age. For the older patient, a trial of an oral elemental diet may be effective. Improvement on an elemental diet may be the result of the removal of multiple offending dietary antigens. For those patients who do not respond to dietary changes, oral cromolyn sodium has produced a good response in selected patients.⁶⁹ Some patients, however, require intermittent low-dose corticosteroid therapy to obtain a sustained remission of symptoms.

Small Intestine

Milk- and Soy-Induced Enteropathy

Small intestinal injury is associated with a variety of clinical syndromes of food sensitivity. It is a site of injury for the three most common dietary antigens of childhood: cow's milk, soy, and gluten. Small intestinal injury due to cow's milk protein has been described to produce the following

clinical syndromes: (1) anemia and iron deficiency secondary to chronic gastrointestinal blood loss in infants drinking homogenized, pasteurized cow's milk,⁷⁰ (2) gastrointestinal protein loss, edema, and hypoalbuminemia associated with other symptoms of atopy,^{58, 68} and (3) chronic diarrhea and malabsorption leading to significant failure-to-thrive.^{71, 72} Cow's milk protein sensitivity has been implicated in the prolongation of diarrhea after acute infectious enteritis.⁷³⁻⁷⁵ In addition, soy protein also has been implicated in the genesis of malabsorption and chronic diarrhea.⁷⁶ Concomitant soy and cow's milk intolerance may occur in about one third of affected children.^{77, 78}

Common to all these clinical syndromes is the presence of small intestinal mucosal injury of varying severity. This may lead to variable degrees of diarrhea, malabsorption, and intestinal losses of protein. The initial approach to patients with suspected protein-sensitive enteritis is based on ruling out other potential causes for malabsorption and diarrhea. Stools should be examined for fecal leukocytes and ova and parasites, and stool cultures should be obtained. Simultaneously, the patient can be placed on a casein hydrolysate formula to initiate nutritional repletion. A good response to an elemental formula may obviate the need for extensive evaluations for other causes of malabsorption. In the sickest patients, parenteral hyperalimentation may be necessary if adequate calories cannot be delivered enterally.

Ultimately, the diagnostic approach is based on demonstrating morphologic and/or functional impairment of the small intestinal mucosa and the response to oral challenge. The presence of mucosal injury has led some authors to suggest that the use of prechallenge and postchallenge small intestinal mucosal biopsies may obviate the need to attempt to fulfill Goldman's criteria. Shiner et al.⁷⁹ compared prechallenge and postchallenge biopsy specimens in four infants and were able to document postchallenge jejunal mucosal abnormalities (partial to severe villous atrophy) in all four infants. The lack of a clinical response to oral challenge in these infants suggested that mucosal biopsy may be more sensitive than clinical criteria. Using the 1-hour d-xylose test as a noninvasive estimation of small intestinal mucosal injury, Morin et al.⁸⁰ showed a significant reduction in the postchallenge 1-hour d-xylose level in infants with cow's milk protein intolerance.

Other authors, however, have questioned the sensitivity and specificity of both mucosal biopsy and the d-xylose test in the diagnosis of cow's milk protein sensitive enteropathy.⁸¹⁻⁸³ Several infants with abnormal postchallenge biopsy specimens, but without clinical symptoms at the time of challenge, were maintained on a cow's milk-containing diet.⁸³ These infants continued to demonstrate adequate growth and weight gain in spite of their response to oral challenge. Conflicting studies like these may relate to several factors. Lack of standardization of an oral challenge dose may lead to differences in the timing of subsequent mucosal damage and clinical symptoms. The description by Kuitunen et al.⁷² of patients developing

symptoms after 3 to 4 weeks of a continuous challenge would have led to this subgroup of infants not being classified as having food sensitivity by Goldman's criteria (48 hours of clinical observation). The delayed development of mucosal abnormalities might be missed if the biopsy is performed soon after oral challenge. In addition, these mucosal abnormalities may have a patchy distribution within the small bowel⁸⁴ and, thus, normal biopsy specimens may be obtained in sensitive subjects due to sampling error. Several authors have shown that the susceptibility to an adverse reaction to cow's milk-containing formula decreases with advancing age, possibly reflecting maturation of the mechanisms affecting oral tolerance and intestinal permeability.^{72, 85, 86} Thus, some infants' sensitivity may spontaneously have resolved by the time of diagnostic testing.

With these exceptions, most investigators currently use an approach based on initially observing the response to milk withdrawal (Fig 1). Diagnostic challenge is usually performed after a period of at least 1 month to allow adequate healing and, when possible, closer to 1 year of age to allow for possible spontaneous resolution of disease. Because some reactions may be severe, challenge is usually performed in hospital with close observation to document the clinical response. Previous small intestinal injury may result in persistent lactase deficiency. Thus, a lactose breath hydrogen

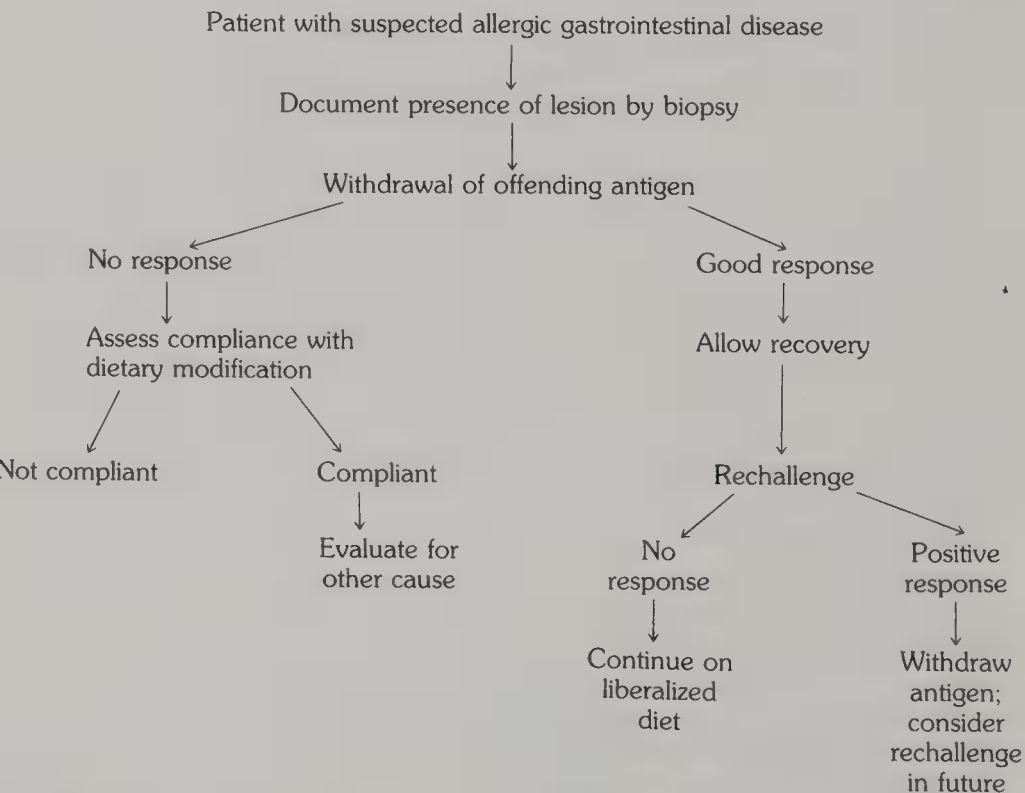


FIG 1.
Approach to the patient with suspected food sensitivity.

test may be performed prior to the challenge to rule out lactose malabsorption as a potential confounding variable. Challenge is performed with increasing dosages of the suspected antigen until a clinical response is observed. Alternatively, capsules with dried nonfat milk can be used for double-blind challenge in situations where equivocal results are obtained by unblinded challenge.²⁸ If no clinical response occurs then the child is continued on an unrestricted diet with close outpatient follow-up to document continued well-being and weight gain. Small intestinal biopsy plays a limited role, usually employed in the initial evaluation of the patient, to document the presence of a small intestinal mucosal lesion in the child presenting with either protein-losing enteropathy or malabsorption.

Gluten-Sensitive Enteropathy

Gluten-sensitive enteropathy, or celiac sprue, is the model for gastrointestinal manifestations of food sensitivity. While the clinical features and putative pathophysiologic mechanisms involved in celiac disease have been extensively reviewed elsewhere,⁸⁷ several points deserve mention for comparison and contrast with cow's milk- and soy protein-induced small intestinal damage. Similar to milk-induced disease, celiac disease commonly presents with symptoms and signs of malabsorption including diarrhea, weight loss, and abdominal distension. However, the presentation of celiac disease is not confined to infancy. The recognition of celiac disease in older patients may be associated with a wide variety of presenting symptoms including short stature, chronic constipation, behavioral disturbances, or vitamin deficiency syndrome.^{87, 88}

For both celiac disease and milk-induced enteropathy the precise mechanisms of intestinal damage remain to be elucidated. Either a direct toxic effect of gluten or a gluten-induced activation of local mucosal humoral and cellular immune responses has been implicated in the pathogenesis of celiac disease.⁸⁹ Similarly, abnormal humoral and cell-mediated immune responses to cow's milk and soy have been found in patients with milk-sensitive enteropathy.^{90, 91} In addition, histologic studies have detected similar histologic and morphometric changes in the two diseases.^{92, 93} However, clinical and epidemiologic information suggest that there are different factors that predispose to these two conditions. Familiar clustering of celiac disease as well as a recognized association with the HLA antigens B8 and DW3 support a role for genetic factors in the susceptibility to gluten.⁸⁹ The tendency for milk- and soy-induced enteropathy to occur in infants and to resolve with age favors a primary role for development and maturational factors being of pathogenetic significance. This is supported by the previously mentioned alterations in oral tolerance and intestinal permeability which occur during infancy.

A second aspect of celiac disease relates to the varied responses to dietary gluten that have been described. Walker-Smith⁹⁴ reported a syndrome of transient gluten intolerance in infancy leading to diarrhea and malabsorption which, similar to cow's milk enteropathy, resolves with age.

Several subjects have also been described who have had abdominal pain and diarrhea that responded to gluten withdrawal, recurred on rechallenge, but was not associated with the typical histologic changes of celiac sprue.^{95, 96} Finally, malabsorption of the carbohydrate component of wheat, associated with an elevated breath hydrogen concentration, has been demonstrated in normal subjects.⁹⁷ This may cause symptoms of malabsorption without an associated adverse immunologic response to gluten. Thus, for a given dietary antigen, variability in the local immune response of the human gastrointestinal tract may occur.

The tendency for milk sensitivity to resolve, in contrast to celiac disease, which is a lifelong problem, has led to a more rigorous approach to the diagnosis of celiac disease. Because of the focality of the lesion and the timing of clinical abnormalities after oral challenge, the benefit of small intestinal biopsy is controversial in the management of milk sensitivity. In contrast, most authors recommend the use of three biopsies to establish the diagnosis of celiac disease.⁸⁷ An initial biopsy specimen documents the presence of a diffuse small intestinal mucosal lesion. The second biopsy specimen verifies improvement on a gluten-free diet, and the third biopsy specimen documents mucosal injury after a gluten challenge. Unlike the patchy lesion of milk protein-induced disease, in celiac disease the mucosa is uniformly involved with a lesion characterized by villous atrophy, crypt hyperplasia, and a gradient of severity that decreases distally along the small intestine. Thus, mucosal biopsy of the proximal small intestine (usually performed at the ligament of Treitz) avoids sampling error. Similar to milk-induced disease, however, variability has been observed in the timing between oral challenge and clinical or histologic relapse.⁹⁸

The rigorous diagnostic approach to celiac disease previously described is the result of a variety of factors. While several nutritional alternatives exist as substitutes for cow's milk or soy formula feeding in infancy, the treatment of celiac disease requires lifelong adherence to a gluten-free diet. Poor compliance is often associated with relapse of malabsorptive symptoms. In addition, a variety of gastrointestinal (ulcerative jejunitis), immune (splenic atrophy), and possibly malignant complications have been linked to celiac disease.⁹⁹⁻¹⁰¹ Thus, the diagnosis of celiac disease must be established prior to committing the patient to lifelong dietary restriction.

Colon

Colitis in infants, as a manifestation of food sensitivity, is a well-recognized clinical occurrence.¹⁰² It generally presents early during the first year of life with bloody diarrhea that may be quite severe. Weight loss and failure-to-thrive may exist if enteritis is associated.^{103, 104} Colitis has been identified in infants fed milk protein, soy protein, and recently, breast milk.¹⁰⁴⁻¹⁰⁶

Postulated causes for milk- and soy-induced colitis are similar to those for the often associated small bowel lesion. Colonic permeability to macromolecules is known to be increased in neonatal, compared with adult, animals.¹⁰⁷ Active inflammation enhances colonic permeability to macro-

molecules,¹⁰⁸ and thus it is conceivable that an initial inflammatory or infectious insult may allow uptake and sensitization to dietary antigens. Similar to protein-induced small intestinal disease, the frequency and severity of milk-induced colitis decrease with advancing age, suggesting a critical role for developmental factors in the pathogenesis of this illness.

The initial approach to these patients begins with excluding infectious causes for colitis. Proctoscopy may reveal mucosal friability, erythema, edema, ulceration, or frank hemorrhage.^{102, 106} This may be useful for establishing that the diarrhea and bleeding are due to colonic inflammation. Biopsy specimens usually reveal acute and/or chronic inflammation, which may be focal, and frequently also show an increase in mucosal eosinophils and plasma cells.^{102, 106, 109, 110} Hirschsprung's disease, which may occasionally present with a colitic syndrome, can also be eliminated by biopsy. As with other gastrointestinal disease due to food sensitivity, the proof that the colonic inflammation is related to an abnormal response to a dietary antigen rests on the demonstration that a similar response occurs following diagnostic challenge. This is usually performed after the suspected dietary antigen has been removed from the diet for a time to allow colonic inflammation to resolve. As with other food sensitivity-related gastrointestinal disease in infants, sensitivity often resolves by the end of the first or second year of life.¹⁰⁴

Summary

A wide range of clinical syndromes exist that are related to adverse reactions to dietary proteins and that affect predominantly the gastrointestinal tract of infants and children. Experimental data suggest a critical role for developmental alterations affecting intestinal permeability and the mucosal immune response that predispose to these conditions. The diagnostic and therapeutic approach to these disorders varies depending on the nature of the presumed offending antigen, the anatomic site affected, the severity of the inflammatory process, and the implications for future dietary and medical management. Ultimately, the proof that a particular dietary antigen is responsible is dependent on observing the response to oral challenge.

References

1. Stern M, Walker WA: Food allergy and intolerance. *Pediatr Clin North Am* 1985; 32:471-492.
2. McCarty EP, Frick OL: Food sensitivity: Keys to diagnosis. *J Pediatr* 1983; 102:645-652.
3. Bock SA: Food sensitivity. *Am J Dis Child* 1980; 134:973-982.
4. Metcalfe DD: Food hypersensitivity. *J Allergy Clin Immunol* 1984; 73:749-762.
5. Atkins FM, Metcalfe DD: The diagnosis and treatment of food allergy. *Annu Rev Nutr* 1984; 4:233-255.

6. Mowat AM: The regulation of immune responses to dietary protein antigens. *Immunol Today* 1987; 8:93-98.
7. Miller SD, Hanson DG: Inhibition of specific immune responses by feeding protein antigens: IV. Evidence for tolerance and specific active suppression of cell-mediated immune response to ovalbumin. *J Immunol* 1979; 123:2344-2350.
8. Richman LK, Chiller JM, Brown WR, et al: Enterically induced immunologic tolerance: I. Induction of suppressor T lymphocytes by intragastric administration of soluble proteins. *J Immunol* 1978; 121:2429-2434.
9. Hanson DG: Ontogeny of orally induced tolerance to soluble proteins in mice: I. Priming and tolerance in newborns. *J Immunol* 1981; 127:1518-1524.
10. Strobel S, Ferguson A: Immune responses to fed protein antigens in mice: 3. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr Res* 1984; 18:588-594.
11. Stokes CR, Newby TJ, Bourne FJ: The influence of oral immunization on local and systemic immune responses to heterologous antigens. *Clin Exp Immunol* 1983; 52:399-406.
12. Strobel S, Mowat AM, Drummond HE, et al: Immunological responses to fed protein antigens in mice: II. Oral tolerance for CMI is due to activation of cyclophosphamide-sensitive cells by gut processed antigen. *Immunology* 1983; 49:451-456.
13. Muckerheide A, Pesce AJ, Michael JG: Immunosuppressive properties of a peptic fragment of BSA. *J Immunol* 1977; 119:1340-1345.
14. Block KJ, Block KB, Sterns M, et al: Intestinal uptake of macromolecules: VI. Uptake of protein antigen in vivo in normal rats and rats infected with *Nippostrongylus brasiliensis* or subjected to mild systemic anaphylaxis. *Gastroenterology* 1979; 77:1039-1044.
15. Walker WA, Wu M, Isselbacher KJ, et al: Intestinal uptake of macromolecules: IV. The effect of pancreatic duct ligation on the breakdown of antigen and antigen-antibody complexes on the intestinal surface. *Gastroenterology* 1975; 69:1223-1229.
16. Pang KY, Walker WA, Bloch KJ: Intestinal uptake of macromolecules: difference in distribution and degradation of protein antigen in control and immunised rats. *Gut* 1981; 22:1018-1024.
17. Forstner G, Sturgess J, Forstner J: Malfunction of intestinal mucus and mucus production. *Adv Exp Med Biol* 1976; 89:349.
18. Bresson JL, Pang K, Udall J, et al: Evidence for increased enterotoxin binding to newborn microvillus membranes: a possible explanation for enhanced toxigenic diarrhea in infancy. *Gastroenterology* 1980; 78:1145.
19. Walker WA, Isselbacher KJ: Intestinal antibodies. *N Engl J Med* 1977; 297:767-773.
20. Walker WA, Wu M, Bloch KJ: Stimulation by immune complexes of mucus release from goblet cells of the rat small intestine. *Science* 1977; 197:370-372.
21. Walker WA, Bloch KJ: Uptake of antigen-antibody complexes prepared in antibody or antigen excess by normal rat intestine in vitro. *Gastroenterology* 1976; 70:948.
22. Udall JN, Pang K, Fritze L, et al: Development of gastrointestinal mucosal barrier: I. The effect of age on intestinal permeability to macromolecules. *Pediatr Res* 1981; 15:241-244.

23. Pang KY, Bresson JL, Walker WA: Development of the gastrointestinal mucosal barrier: V. Comparative effect of calcium binding on microvillus membrane structure in newborn and adult rats. *Pediatr Res* 1983; 17: 856-861.
24. Stern MS, Pang KY, Walker WA: Food proteins and gut mucosal barrier: II. Differential interaction of cow's milk proteins with the mucous coat and the surface membrane of adult and immature rat jejunum. *Pediatr Res* 1984; 18:1252-1257.
25. Selner JC, Merrill DA, Claman HN: Salivary immunoglobulin and albumin: Development during the newborn period. *J Pediatr* 1968; 72:685-689.
26. Burgio GR, Lanzavecchia A, Plebani A, et al: Ontogeny of secretory immunity: Levels of secretory IgA and natural antibodies in saliva. *Pediatr Res* 1980; 14:1111-1114.
27. Perkkio M, Savilahti E: Time of appearance of immunoglobulin-containing cells in the mucosa of the neonatal intestine. *Pediatr Res* 1980; 14:953-955.
28. Bock SA, Lee W-Y, Remigio LK, et al: Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978; 62:327-334.
29. Goldman AS, Anderson DW, Sellers WA, et al: I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963; 32:425-443.
30. Bjorksten B, Ahlstedt S, Bjorksten F, et al: Immunoglobulin E and immunoglobulin G4 antibodies to cow's milk in children with cow's milk allergy. *Allergy* 1983; 38:119-124.
31. Schwartz HR, Nerurkar LS, Spies JR, et al: Milk hypersensitivity: RAST studies using new antigens generated by pepsin hydrolysis of beta-lactoglobulin. *Ann Allergy* 1980; 45:242-245.
32. Bellanti JA, Nerurkar LS, Willoughby JW: Measurements of plasma histamine in patients with suspected food hypersensitivity. *Ann Allergy* 1981; 47:260-263.
33. Sampson HA, Jolie PL: Increased plasma histamine concentrations after food challenges in children with atopic dermatitis. *N Engl J Med* 1984; 311:372-376.
34. May CD: High spontaneous release of histamine in vitro from leukocytes of persons hypersensitive to food. *J Allergy Clin Immunol* 1976; 58:432-437.
35. McLaughlan P, Coombs RRA: Latent anaphylactic sensitivity of infants to cow's milk proteins. *Clin Allergy* 1983; 13:1-9.
36. Bock SA, Buckley J, Holst A, et al: Proper use of skin tests with food extracts in diagnosis of hypersensitivity to food in children. *Clin Allergy* 1977; 7:375-383.
37. Sampson HA, Albergo R: Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 1984; 74:26-33.
38. Leary HL, Halsey JF: An assay to measure antigen-specific immune complexes in food-allergy patients. *J Allergy Clin Immunol* 1984; 74:190-195.
39. Martin ME, Guthrie LA, Bock SA: Serum complement changes during double-blind food challenges in children with a history of food sensitivity. *Pediatrics* 1984; 73:532-537.
40. Matthews TS, Soothill JF: Complement activation after milk feeding in children with cow's milk allergy. *Lancet* 1970; 2:893-895.
41. Ashkenazi A, Levin S, Idar D, et al: In vitro cell-mediated immunologic assay for cow's milk allergy. *Pediatrics* 1980; 66:399-402.
42. Iyngkaran N, Yadav M, Balabaskaran S, et al: In vitro diagnosis of cow's milk

- protein sensitive enteropathy by organ culture method. *Gut* 1980; 22:199–202.
43. Goldman AS, Sellars WA, Halpern SR, et al: Milk allergy: II. Skin testing of allergic and normal children with purified milk proteins. *Pediatrics* 1963; 32:572–579.
44. Saperstein S, Anderson DW, Goldman AS, et al: Milk allergy: III. Immunologic studies with sera from allergic and normal children. *Pediatrics* 1963; 32:580–587.
45. Chua YY, Bremner K, Lakdawalla N, et al: In vivo and in vitro correlates of food allergy. *J Allergy Clin Immunol* 1976; 58:299–307.
46. Sinatra FR, Merritt RJ: Iatrogenic kwashiorkor in infants. *Am J Dis Child* 1981; 135:21–23.
47. Lloyd-Still JD: Chronic diarrhea of childhood and the misuse of elimination diets. *J Pediatr* 1979; 95:10–13.
48. Warner J, Hathaway MJ: Allergic form of Meadow's syndrome (Munchausen by proxy). *Arch Dis Child* 1984; 59:151–156.
49. Anderson LB, Dreyfuss EM, Logan J, et al: Melon and banana sensitivity coincident with ragweed pollinosis. *J Allergy* 1970; 45:310–319.
50. Ferguson R, Basu MK, Asquith P, et al: Jejunal mucosal abnormalities in patients with recurrent aphthous ulceration. *Br Med J* 1975; 1:11–13.
51. Wray D: Gluten-sensitive recurrent aphthous stomatitis. *Dig Dis Sci* 1981; 26:737–740.
52. Anderson JA, Jackson CE, Krull EA, et al: Hypersensitive furrowed mouth. *Clin Proc CHNMC* 1980; 36:269–286.
53. Huntley CC, Bowers GW, Vann RL: Allergic protein-losing gastroenteropathy: report of an unusual case. *South Med J* 1970; 63:917–922.
54. Lehner T: Immunological aspects of recurrent oral ulceration and Behcet's syndrome. *J Oral Pathol* 1978; 7:424–430.
55. Ship II: Inheritance of aphthous ulcers in the mouth. *J Dent Res* 1965; 44:837–844.
56. Ship II: Epidemiologic aspects of recurrent aphthous ulcerations. *Oral Surg* 1972; 33:400–406.
57. Thomas HC, Ferguson A, McLennan JG, et al: Food antibodies in oral disease: A study of serum antibodies to food proteins in aphthous ulceration and other oral diseases. *J Clin Pathol* 1973; 26:371–374.
58. Waldmann TA, Wochner RD, Laster L, et al: Allergic gastroenteropathy: A cause of excessive gastrointestinal protein loss. *N Engl J Med* 1967; 276:761–769.
59. Caldwell JH, Mekhjian HS, Hurtubise PE, et al: Eosinophilic gastroenteritis with obstruction: Immunologic studies of seven patients. *Gastroenterology* 1978; 74:825–829.
60. McNabb PC, Fleming CR, Higgins JA, et al: Transmural eosinophilic gastroenteritis with ascites. *Mayo Clin Proc* 1979; 54:119–122.
61. Katz AJ, Goldman H, Grand RJ: Gastric mucosal biopsy in eosinophilic (allergic) gastroenteritis. *Gastroenterology* 1977; 73:705–709.
62. Dobbins JW, Sheahan DG, Behar J: Eosinophilic gastroenteritis with esophageal involvement. *Gastroenterology* 1977; 72:1312–1316.
63. Haberkern CM, Christie DL, Haas JE: Eosinophilic gastroenteritis presenting as ileocolitis. *Gastroenterology* 1978; 74:896–899.
64. Tedesco FJ, Huckaby CB, Hamby-Allen M, et al: Eosinophilic ileocolitis: Ex-

- panding spectrum of eosinophilic gastroenteritis. *Dig Dis Sci* 1981; 26:943–948.
65. Caldwell JH, Teenenbaum JI, Bronstein HA: Serum IgE in eosinophilic gastroenteritis: Response to intestinal challenge in two cases. *N Engl J Med* 1975; 292:1388–1390.
66. Caldwell JH, Sharma HM, Hurtubise PE, et al: Eosinophilic gastroenteritis in extreme allergy: Immunopathological comparison with nonallergic gastrointestinal disease. *Gastroenterology* 1979; 77:560–564.
67. Teele RL, Katz AJ, Goldman H, et al: Radiologic features of eosinophilic gastroenteritis (allergic gastroenteropathy) of childhood. *AJR* 1979; 132:575–580.
68. Katz AJ, Twarog FJ, Zeiger RS, et al: Milk-sensitive and eosinophilic gastroenteropathy: similar clinical features with contrasting mechanisms and clinical course. *J Allergy Clin Immunol* 1984; 74:72–78.
69. Heatley RV, Harris A, Atkinson M: Treatment of a patient with clinical features of both eosinophilic gastroenteritis and polyarteritis nodosa with oral sodium cromoglycate. *Dig Dis Sci* 1980; 25:470–472.
70. Wilson JF, Lahey ME, Heiner DC: Studies on iron metabolism: V. Further observations on cow's milk-induced gastrointestinal bleeding in infants with iron-deficiency anemia. *J Pediatr* 1974; 84:335–344.
71. Iyngkaran N, Robinson MJ, Prathap K, et al: Cow's milk protein-sensitive enteropathy: Combined clinical and histological criteria for diagnosis. *Arch Dis Child* 1978; 53:20–26.
72. Kuitunen P, Visakorpi JK, Savilahti E, et al: Malabsorption syndrome with cow's milk intolerance: clinical findings and course in 54 cases. *Arch Dis Child* 1975; 50:351–356.
73. Iyngkaran N, Davis K, Robinson MJ, et al: Cow's milk protein sensitive enteropathy: An important contributing cause of secondary sugar intolerance in young infants with acute infective enteritis. *Arch Dis Child* 1979; 54:39–43.
74. Iyngkaran N, Robinson MJ, Sumithran E, et al: Cow's milk protein-sensitive enteropathy: An important factor in prolonging diarrhea of acute infective enteritis in early infancy. *Arch Dis Child* 1978; 53:150–153.
75. Iyngkaran N, Abdin Z, Davis K, et al: Acquired carbohydrate intolerance and cow milk protein-sensitive enteropathy in young infants. *J Pediatr* 1979; 95:373–378.
76. Ament ME, Rubin CE: Soy protein: another cause of the flat intestinal lesion. *Gastroenterology* 1972; 62:227–234.
77. Whittington PF, Gibson R: Soy protein intolerance: four patients with concomitant cow's milk intolerance. *Pediatrics* 1977; 59:730–732.
78. Perkkio M, Savilahti E, Kuitunen P: Morphometric and immunohistochemical study of jejunal biopsies from children with intestinal soy allergy. *Eur J Pediatr* 1981; 37:63–69.
79. Shiner M, Ballard J, Brook CGD, et al: Intestinal biopsy in the diagnosis of cow's milk protein intolerance without acute symptoms. *Lancet* 1975; 2:1060–1063.
80. Morin CL, Buts J-P, Weber A, et al: One-hour blood-xylose test in diagnosis of cow's milk protein intolerance. *Lancet* 1979; 1:1102–1104.
81. Berg NO, Jakobsson I, Lindberg T: Do pre- and postchallenge small intestinal biopsies help to diagnose cow's milk protein intolerance? *Acta Paediatr Scand* 1979; 68:657–661.

82. Iyngkaran N, Abidin Z: One-hour blood xylose in the diagnosis of cow's milk protein-sensitive enteropathy. *Arch Dis Child* 1982; 57:40-43.
83. Sumithran E, Iyngkaran N: Is jejunal biopsy really necessary in cow's milk protein intolerance? *Lancet* 1977; 2:1122-1123.
84. Walker-Smith JA: Jejunal biopsy in cow's milk protein intolerance. *Lancet* 1977; 2:1237.
85. Hill DJ, Davidson GP, Cameron DJS, et al: The spectrum of cow's milk allergy in childhood: Clinical, gastroenterological, and immunologic studies. *Acta Paediatr Scand* 1979; 68:847-852.
86. Jakobsson I, Lindberg T: A prospective study of cow's milk protein intolerance in Swedish infants. *Acta Paediatr Scand* 1979; 68:853-859.
87. Trier JS, Falchuk ZM, Carey MC, et al: Celiac sprue and refractory sprue. *Gastroenterology* 1978; 75:307-316.
88. Cacciari E, Salardi S, Lazzari R, et al: Short stature and celiac disease: A relationship to consider even in patients with no gastrointestinal tract symptoms. *J Pediatr* 1983; 103:708-711.
89. Mike N, Asquith P: Gluten toxicity in coeliac disease and its role in other gastrointestinal disorders, in Brostoff J, Challacombe SJ (eds): *Food Allergy and Intolerance*. London, Bailliere Tindall, 1987, pp 521-548.
90. McDonald PJ, Goldblum RM, Van Sickle GJ, et al: Food protein-induced enterocolitis: Altered antibody response to ingested antigen. *Pediatr Res* 1984; 18:751-755.
91. Van Sickle GJ, Powell GK, McDonald PJ, et al: Milk- and soy protein-induced enterocolitis: Evidence for lymphocyte sensitization to specific food proteins. *Gastroenterology* 1985; 88:1915-1921.
92. Kosnai I, Kuitunen P, Savilahti E, et al: Mast cells and eosinophils in the jejunal mucosa of patients with intestinal cow's milk allergy and celiac disease of childhood. *J Pediatr Gastroenterol Nutr* 1984; 3:368-372.
93. Marsh MN, Hinde J: Inflammatory component of celiac sprue mucosa: I. Mast cells, basophils, and eosinophils. *Gastroenterology* 1985; 89:92-101.
94. Walker-Smith J: Transient gluten intolerance. *Arch Dis Child* 1970; 45:523-526.
95. Doherty M, Barry RE: Gluten-induced mucosal changes in subjects without overt small bowel disease. *Lancet* 1981; 1:517-520.
96. Cooper BT, Holmes GKT, Ferguson R, et al: Gluten-sensitive diarrhea without evidence of celiac disease. *Gastroenterology* 1980; 79:801-806.
97. Anderson IH, Levine AS, Levitt MD: Incomplete absorption of the carbohydrate in all-purpose wheat flour. *N Engl J Med* 1981; 304:891-892.
98. McNicholl B, Egan-Mitchell B, Fottrell PF: Variability of gluten intolerance in treated childhood coeliac disease. *Gut* 1979; 20:126-132.
99. Bayless TM, Kapelowitz RF, Shelley WM, et al: Intestinal ulceration: A complication of celiac disease. *N Engl J Med* 1967; 276:996-1002.
100. O'Grady JG, Stevens FM, Harding B, et al: Hyposplenism and gluten-sensitive enteropathy: Natural history, incidence, and relationship to diet and small bowel morphology. *Gastroenterology* 1984; 87:1326-1331.
101. Selby WS, Gallagher ND: Malignancy in a 19-year experience of adult celiac disease. *Dig Dis Sci* 1979; 24:684-688.
102. Jenkins HR, Pincott JR, Soothill JF, et al: Food allergy: The major cause of infantile colitis. *Arch Dis Child* 1984; 59:326-329.
103. Powell GK: Enterocolitis in low-birth-weight infants associated with milk and soy protein intolerance. *J Pediatr* 1976; 88:840-844.

104. Powell GK: Milk- and soy-induced enterocolitis of infancy: Clinical features and standardization of challenge. *J Pediatr* 1978; 93:553-560.
105. Halpin TC, Byrne WJ, Ament ME: Colitis, persistent diarrhea, and soy protein intolerance. *J Pediatr* 1977; 91:404-407.
106. Lake AM, Whittington PF, Hamilton SR: Dietary protein-induced colitis in breast-fed infants. *J Pediatr* 1982; 101:906-910.
107. Seidman EG, Hanson DG, Ely I, et al: Macromolecular uptake from the colon: Effect of age on permeability in rabbits. *Gastroenterology* 1985; 88:1579.
108. Seidman EG, Hanson DG, Walker WA: Increased permeability to polyethylene glycol 4000 in rabbits with experimental colitis. *Gastroenterology* 1986; 90:120-126.
109. Goldman H, Proujansky R: Allergic proctitis and gastroenteritis in children: Clinical and mucosal biopsy features in 53 cases. *Am J Surg Pathol* 1986; 10:75-86.
110. Sherman MP, Cox KL: Neonatal eosinophilic colitis. *J Pediatr* 1982; 100:587-589.

Indoor Air Pollutants

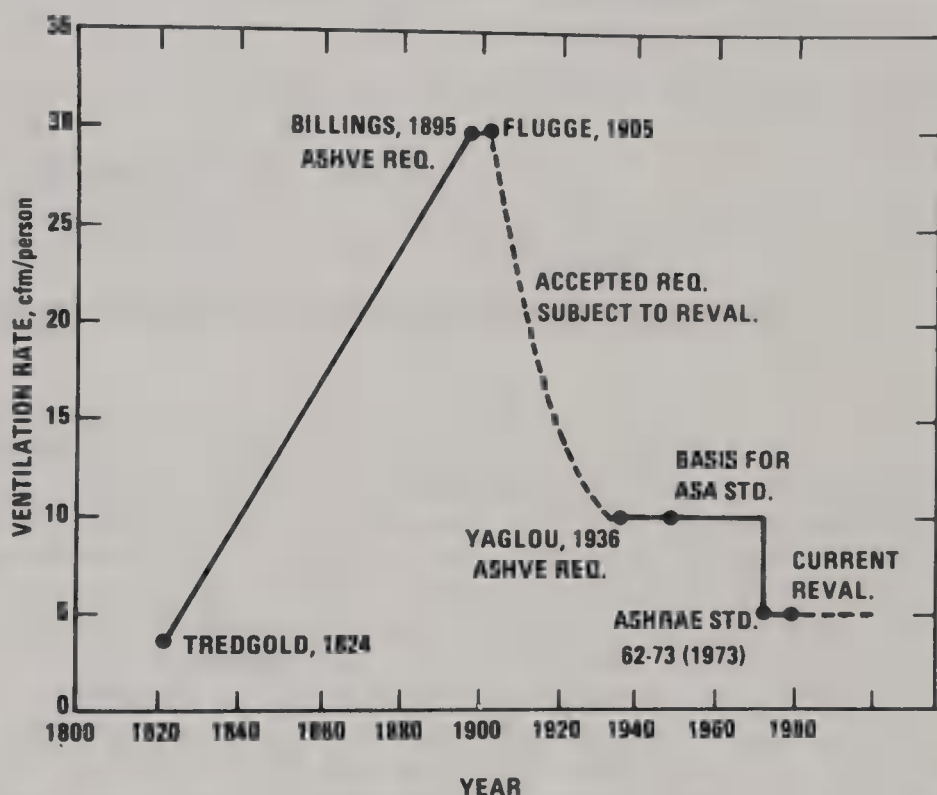
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A full generation of children has grown up since the passage of the Clean Air Act of 1970. National outdoor air quality was rapidly and significantly improved by the concurrence of regulatory enforcement and the economic crisis of rising costs for fossil fuels. Simultaneous and progressive concern with the deteriorating quality of indoor air related to energy conservation techniques of construction, new building materials, alternate modes of thermal energy, and the continuing introduction of synthetic chemicals as household products. In only 10 years, the wood-burning stove went from a symbol of ecologic adaptation to a respiratory hazard. Parents now turn to their pediatricians with complex questions concerning the detection, elimination, and health effects of indoor pollutants. To assist in the response, this is a review of the major categories of pollutants, their effects on the health of children, and the current estimates of latent risk.

Historical Perspective

Concern with household ventilation dates to antiquity and longstanding regulations predate the new emphasis spelled out by the Radon, Gas, and Indoor Air Quality Research Act of the 1986 Superfund Bill (PL 99-499). In 1824 Tredgold¹ proposed an exchange of 4 cubic feet per minute (cfm) per person between indoor and outdoor air to prevent stuffiness and maintain the CO₂ concentration below 0.1% (Fig 1). By 1887 Carnelley et al.² proposed a microbial level of below 12 microorganisms per liter. In 1895, The American Society of Heating and Ventilation Engineers adopted the recommendations of Billings³ of an exchange of 30 cfm, incorporated in the building codes of 22 states by 1925. In 1936 this was downgraded to 10 cfm as adequate to control body odors. In 1975 an energy-saving standard of 5 cfm was proposed by the American Society of Heating Refrigeration and Air-Conditioning Engineers and was subsequently incorporated into the building codes of 45 states. The 1975 standard led to a gross reduction of the air supplied to building occupants and to widespread complaints of the "tight building syndrome" in both commercial buildings and residences.

**FIG 1.**

Historical development of ASHRAE standards.^{1-3, 133} (From Mage DT, Gammage RB: Evaluation of changes in indoor air quality occurring over the past several decades, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

As an example, newer homes have a median of 0.5 air changes per hour as compared with the 0.9 exchanges in older homes.⁴ The significance of these decreases in ventilation must be considered in association with the perception that people spend more time indoors. One of the few facts supporting this impression is the increase in the average daily running time of a TV set from 4 to 5 hours in 1950 to 7 hours in 1983.⁵ Whether this represents a trend, children spend over 75% of their time indoors and most spend at least 40 hours a week in "tight buildings." Just as the impact of all diseases, from malnutrition to mental illness, is greater than the sum of the parts, so is the stuffy building syndrome. The multiple physical complaints of occupants of stuffy buildings have never been really explained by any single component, not even by psychologic distress and hysteria.⁶ Despite the almost complete lack of information on the synergistic, additive, and antagonistic effects of composite exposures, there are sufficient data concerning individual pollutants to merit the attention of all concerned with environmental effects on child health. This review focuses on the ma-

for groups: combustion gases, environmental tobacco smoke, asbestos, volatile organic gases, radon and nonionizing radiation, pathogens, and allergens.

Combustion Gases

The combustion of fossil fuels and of wood and plant materials results in the emission of complex mixtures of organic and inorganic gaseous and particulate pollutants. The primary contaminants are three products of incomplete combustion: carbon monoxide, nitrogen dioxide, and sulfur dioxide. The current National Ambient Air Quality Standards (NAAQS) for these provide a reference for interpreting subsequent data on indoor air quality (Table 1). The NAAQS levels are maximal or unhealthful levels, and the 1- to 24-hour averages are not to be exceeded more than once a year. Ambient air levels of this magnitude require a public warning for persons with heart or respiratory ailments to reduce physical activity and outdoor exertion.

As regulated criteria pollutants for ambient air, considerable information exists concerning their sources, transport, analysis, biologic disposition, and health effects. The wide range of emission rates for these combustion-re-

TABLE 1.
NAAQS Primary Standard

	$\mu\text{g}/\text{cu m}$	ppm
Sulfur oxides		
Annual arithmetic mean	80	0.030
24-hour mean	365	0.14
Suspended particulate matter		
Annual geometric mean	75	
24-hour mean	260	
Carbon monoxide		
8-hour mean		9.0
1-hour mean		35.0
Ozone		
1-hour mean	235	0.12
Nitrogen oxides		
Annual arithmetic mean	100	0.05
Lead		
90-day arithmetic mean	1.5 (under reevaluation)	

TABLE 2.
Pollutant Emission Rates (mg/hr)*

Source	Appliance Type	NO	NO ₂	CO	SO ₂
Kerosene space heaters	Radiant	0.54-11	16-38	281-542	31-109
Gas space heaters	Convective	2-195 80-4,578	33-530 3-1225	35-635 12-5,004	37-94 ...
Wood heaters		1.2-3.9	1.3-7	70-375	...
Gas appliances	Range (1 burner)	9.5-455	18-430	191-2,700	1.29-1.66
	Oven	30-581	67-270	195-3,564	0.67-1.09

*Adapted from US Department of Energy: *Indoor Air Quality Environmental Information Handbook*, DOE/EV/10450-1. US Department of Energy, 1985.

lated gases from indoor sources as compiled by the Department of Energy in Table 2⁷ indicates the difficulty in developing comparable standards for indoor air.

Carbon Monoxide

CO, the most lethal air pollutant, is at measurable levels in almost all indoor environments, especially in winter, as summarized in several recent reports (Table 3).⁸

In the sampling of the air of 21,543 homes, 4.3% of samples exceeded the current 8-hour NAAQS concentration for CO of 9 ppm (not to be exceeded more than once a year); CO relates primarily to the season and the number of smokers but also to the use of gas heaters and gas ranges.

Carboxyhemoglobin (COHb) levels in peripheral blood are still accepted as an accessible physiologic marker to estimate internal CO burdens due to the combined contribution of endogenously and exogenously derived

TABLE 3.
CO Concentrations of Indoor
Microenvironments*

Site	No. of Sites	CO Concentration (ppm)	
		Mean	SD
Public garage	116	13.46	18.14
Service station or repair facility	125	9.17	9.33
Shopping mall	58	4.90	6.50
Residential garage	66	4.35	7.06
Restaurant	524	3.71	4.35
Office	2,287	3.59	4.18
Store	734	3.23	5.56
Health care facility	351	2.22	4.25
Residence	21,543	2.04	4.06
School	426	1.64	2.76
Church	179	1.56	3.35

*Adapted from Akland GG, Hartwell TD, Johnson TR, et al: Measuring human exposure to carbon monoxide in Washington, DC, and Denver, Colorado, during the winter of 1982-1983. *Environ Sci Technol* 1985; 19:911-918.

CO. The baseline physiologic norms for nonsmokers are estimated as 0.3% to 0.7% COHb. The baseline COHb in smokers typically exceeds 3%. Levels in children and others inhaling environmental tobacco smoke may be intermediate.

For adults with angina, 5.0% COHb is the risk level. Decreased oxygen capacity and decreased work capacity under maximal exercise conditions are demonstrated in healthy young adults at 5.0% COHb with a small decrease at levels as low as 2.3% to 4.3% (Table 4).⁹⁻³⁰

The role of ambient exposure to CO has been postulated but not established in the reduced birth weight of infants exposed in utero to passive tobacco smoke, as has the association of delayed postnatal growth and sudden infant death syndrome. In rats, chronic prenatal exposure at maternal levels of 65 to 150 ppm is associated with delayed learning and cerebellar hypoplasia.³¹ In humans, there are inadequate data defining fetal response to chronic low dose or acute episodic CO except for the grossly adverse effects on the fetus of symptomatic CO poisoning during pregnancy.

TABLE 4.
Statistically Significant Human Health Effects Associated
With Low Level Carbon Monoxide Exposure*

Effects	COHb Concentration (%)
Decreased (~3% to 7%) work time to exhaustion in exercising, young, healthy men ^{10, 11}	2.3 to 4.3
Shortened duration of exercise before onset of pain in angina patients and increased duration of angina attacks ¹²	2.9 to 4.5
Decreased maximal oxygen consumption and exercise time during strenuous exercise in young healthy men ¹³⁻¹⁵	5.0 to 5.5
Statistically significant diminution of visual perception, manual dexterity, ability to learn, performance in complex sensorimotor tasks such as driving ¹⁶⁻²⁶ (but not below 5% COHb ^{16, 27-30})	5.0 to 17.0

*Adapted from US Environmental Protection Agency: Revised Evaluation of Health Effects Associated with Carbon Monoxide Exposure: An Addendum to the 1979 Air Quality Criteria Document for Carbon Monoxide, EPA report no. EPA-600/8-83-033F. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, NTIS, PB85-103471, 1984.

Nitrogen Dioxide

In the presence of indoor sources such as cigarette smoking or gas stoves, NO_2 levels are invariably higher than the outdoor NO_2 . Figure 2 illustrates the diurnal variation in NO_2 in a home with a gas stove with peak levels of $150 \mu\text{g}/\text{cu m}$. Under other circumstances, short-term NO_2 concentrations exceed $1,000 \mu\text{g}/\text{cu m}$ in gas homes during cooking in the kitchen. The effects of NO_2 have been extensively studied^{32, 33} in development of the annual average, outdoor air quality standard (NAAQS) of 0.05 ppm or $100 \mu\text{g}/\text{cu m}$.

The controlled human studies cited in Table 5 have identified asthmatics as highly susceptible to decrements in pulmonary function at levels as low as 0.01 ppm.³⁴⁻⁴⁰ None of these studies are on children and there are no

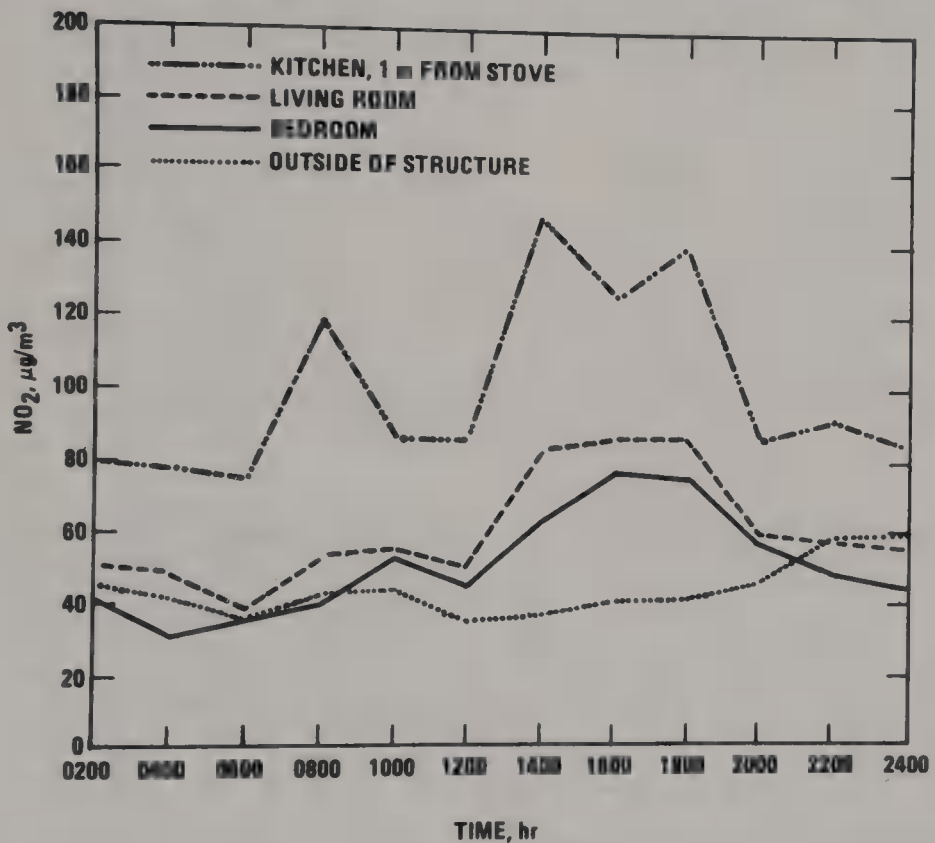


FIG 2.

Diurnal indoor/outdoor pattern for NO_2 in a home as the composite of 6 days of data (spring-summer, 1973).¹³⁴ (From Mage DT, Gammage RB: Evaluation of changes in indoor air quality occurring over the past several decades, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

TABLE 5.
Controlled Human Exposure to NO₂: Pulmonary Function

Study Population	Pollutant/ Concentration	Duration of Exposure and Activity	Pulmonary Effects
20 asthmatics 20 normals ³⁴	NO ₂ 0.1 ppm	1 hr at rest	Decrease in carbachol-induced specific airway conductance in normals ($P < 0.005$) and also in asthmatics ($P < 0.05$)
9 asthmatics ³⁵ 15 normals 15 asthmatics ³⁶ 6 normals ³⁷	NO ₂ 0.1 ppm NO ₂ 0.15 ppm O ₃ 0.15 ppm (NO ₂ + O ₃) 0.15 ppm	1 hr at rest 2-hr intermittent light exercise	No significant effect on airway resistance, FEV ₁ , or volume of isoflow Significant decrease (>5%) in airway conduction/thoracic gas volume with O ₃ and O ₃ -NO ₂ ; borderline decreases with NO ₂
31 asthmatics ³⁸	NO ₂ 0.2 ppm	2-hr intermittent light exercise	No effect on forced expiratory function (FEV ₁) or total respiratory resistance with NO ₂ alone. Borderline increase of methacholine-induced bronchoconstriction in 17 of 21 subjects
15 asthmatics ³⁹	NO ₂ 0.3 ppm	30 min with 10 exercises (VE = 30 L/min)	Decreases in FEV ₁ significantly greater following exercise; increase in airway reactivity to cold air provocation
13 asthmatics ⁴⁰	NO ₂ 0.3 ppm	90 min with 3 intermittent exercises (VE = 45 L/min)	Decrease in FEV ₁ following initial 10-min exercise. After the second and third exercise, specific airway resistance and FEV ₁ decreases significantly greater in NO ₂

controlled studies that adequately test a synergistic effect with other pollutants, as strongly suggested by epidemiologic studies.

In contrast to the controlled exposure studies, the major epidemiologic studies of the effects of gas cooking on lung function in children are still inconclusive (Table 6).⁴¹⁻⁴⁹ Three investigations of large sample sizes⁴¹⁻⁴³ demonstrated statistically significant effects on lung function and five did not.⁴⁴⁻⁴⁹ The current consensus is that the peak NO₂ level of many homes can decrease pulmonary function in asthmatics.

TABLE 6.
Gas Cooking Stoves and Lung Function in Children

Study Population	Lung Function Measure	Results
808 children, 6 to 7 yr, United Kingdom ⁴⁴	PEFR, FEV _{0.75} , FEF ₂₅₋₇₅	No association with NO ₂ levels or presence of gas stove
898 children, 0 to 15 yr, from 441 families, Ohio ^{45, 46}	FVC, FEV _{0.75}	No association with presence of a gas stove
8,120 children, 6 to 10 yr, six US cities ⁴¹	FVC, FEV ₁	Overall reduction of 16 ml and 18 ml respectively, for FEV ₁ and FVC in children from homes with gas stoves
16,689 children, 6 to 13 yr, 7 areas in US ⁴²	FEV _{0.75}	Significant reduction of 19 ml associated with gas stove use in older girls only
676 children, 9 to 11 yr, Arizona ⁴⁷	FEV ₁	No effect of gas stoves on pulmonary level or rate of growth
183 children, 6 to 12 yr, Iowa ⁴⁸	FEV ₁ , FEF ₇₅ , in FEF ₂₅₋₇₅	No change after isoproterenol challenge in children from homes with gas stoves
9,720 children, 6 to 10 yr, six US cities ⁴³	FEV ₁ , FVC	Significant reduction FEV ₁ of 0.6% and FVC of 0.7%; not significant after adjustment for parental education
3,175 children, 5 to 14 yr, Pennsylvania ⁴⁹	FVC, FEV _{0.75} , FEF ₂₅₋₇₅ , Vmax ₇₅ , Vmax ₉₀	No association with use of gas stove

Chronic exposure of the nonasthmatic population to NO_2 is an even more significant cause for concern since animal toxicologic studies unequivocally establish the deleterious effects of acute increases in NO_2 on lung host defenses, biochemistry, and structure.

In multiple epidemiologic studies of the effect of gas stoves that emit NO_2 as well as other pollutants, school-age children have been the primary subjects. Parents have supplied a retrospective history of symptoms and retrospective illness. These studies do not establish either the peak or average exposure to NO_2 . Of eight major studies reporting data on 1,138 to 10,106 children per study, six^{41, 43, 48-53} found an excess of respiratory symptoms or illnesses particularly before age 2 in children living in homes with gas stoves; two did not.^{54, 55} In all studies, the relative risk ratios are borderline and do not provide consistent evidence of the harmful effects of NO_2 exposure at the levels produced by household gas ranges.

Sulfur Dioxide

As shown in Figure 3, there is considerable daily and diurnal variation of SO_2 in a home. The indoor to outdoor ratios of SO_2 exceed unity when there is a smoker in the home or sulfur kerosene is burned in an unvented device. With two kerosene heaters, average indoor levels exceed the outdoor air maximum of $80 \mu\text{g}/\text{cu m}$ in over 50% of homes. Sulfur dioxide exposure is of serious concern to asthmatics since 50% of asthmatic volunteers experience at least a doubling of airway resistance at concentrations below 0.75 ppm SO_2 in exposures as short as 1 minute. There is

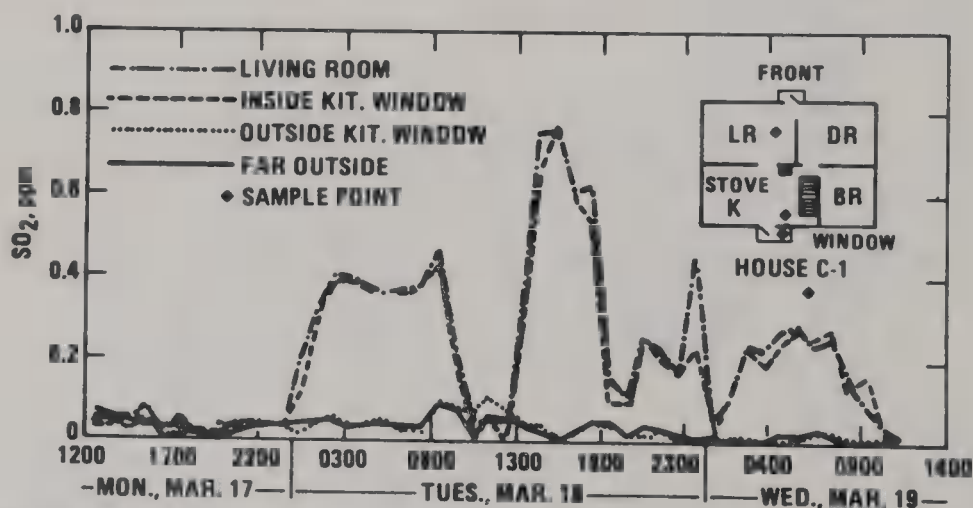


FIG 3.

Time variation of sulfur dioxide in a home.¹³⁵ (From Mage DT, Gammage RB: Evaluation of changes in indoor air quality occurring over the past several decades, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

strong experimental evidence that the deposition of acidic particulates greatly inhibits ciliary clearance. Epidemiologic studies of the sulfur oxides are typically combined with measurements of total suspended particulates (TSPs). Table 7⁵⁶⁻⁶⁰ summarizes five recent studies that demonstrate increased upper or lower respiratory symptoms and decreased pulmonary function in school children and young adults at SO₂ levels of 103 to 280 µg/cu m and TSP levels of 60 to 150 and 200 to 250 µg/cu m.⁵⁶⁻⁶⁰ Particulates vary widely in chemical reactivity. They are generally monitored and distinguished as fine mode (<2.5 µm) and coarse mode (2.5 to 15.0 µm). Fine-mode particulates deposit primarily in the tracheobronchial and pulmonary tract while the coarse-mode particles deposit primarily in the nasopharyngeal region, but the increased water solubility of fine particles, such as chlorine and ammonia, elicits acute upper airway irritation. Honicky et al.⁶¹ attributed the increased incidence of lower respiratory symptoms in 31 children from homes with wood-burning stoves to particulates, but the vapor phase and particulate compositions of wood smoke are at least as complex as tobacco smoke.

Environmental Tobacco Smoke

Environmental tobacco smoke is the primary source of indoor air particulates and defined as the most complex. The major source of environmental tobacco smoke (ETS) is sidestream smoke (SS) emitted from the burning end of the cigarette. Mainstream smoke (MS) is inhaled by the smoker and then exhaled into the room air or emitted as gases that diffuse through the cigarette paper during smoking. As listed in Table 8,^{62, 63} SS contains up to 100 times the MS concentrations of some highly toxic compounds such as carbon monoxide, acrolein, ammonia, carcinogenic nitrosamines and naphthylamine, cadmium, nickel, and polonium. SS emissions are relatively similar across all brands of cigarettes. Both MS and SS are composed of a particulate phase and a vapor phase, the latter being more likely to enter the lungs of the passive smoker. The dominant role of tobacco smoke in the indoor exposure to total suspended particulates is well established.⁶⁴

Exposure to environmental tobacco smoke may begin in utero and continue throughout childhood. The prediction of maximal susceptibility of the developing lung is well established by studies of respiratory symptoms and of lung function in children. Table 9⁶⁵⁻⁷⁰ summarizes six major prospective studies: all showed an increase in bronchitis and pneumonia in the first 2 years of life for children whose parents smoked.

Studies utilizing biologic markers of exposure such as salivary nicotine or urinary cotinine provide more accurate estimates of dose response than the overly simplified classification of smokers per household. Despite this, at least seven major studies^{43, 55, 71-75} show an increasing prevalence of respiratory conditions in school-age children with an increase in the num-

TABLE 7.
Recent Epidemiologic Studies Relating Health Effects to Acute Exposure to Ambient SO₂ and Total Suspended Particulates

Study	24-hr Average Pollutant Levels (µg/cu m)		Results Obtained
	TSPs	SO ₂	
School children, Steubenville, Ohio ⁵⁶	220-420	280-460	Reversible (~2-3 wk), small (2%-3%), but statistically significant decrements in FVC following episodes of 24-hr TSPs 220 to 420 µg/cu m and SO ₂ of 280-460 µg/cu m, but not after "sham" episode with TSP = 160 µg/cu m and SO ₂ = 190 µg/cu m.
School children, Netherlands ⁵⁷	200-250	200-250	Reversible (2-3 wk), small (3%-5%), but statistically significant decrements in pulmonary function measures (FVC, FEV ₁ , MEF) during and after pollution episode when 24-hr TSP and SO ₂ levels up to 200-250 µg/cu m; no effect after pollutants averaged 100-150 µg/cu m.

Six cities longitudinal study ⁵⁸	60–150	...	Increased rates of cough, bronchitis, and lower respiratory disease (without lung function changes) significantly associated with annual-average TSP levels of approximately 30–150 $\mu\text{g}/\text{cu m}$ when analyzed for between city effects; no relation to TSP gradients within individual cities. Effects most clear for highest TSP areas (~ 60 – $160 \mu\text{g}/\text{cu m}$) versus lowest (~ 40 – $60 \mu\text{g}/\text{cu m}$). No association with SO_2 except for cough.
Young adults, Utah, cross-sectional study ⁵⁹	...	115	Increased rates of persistent cough and phlegm with annual-average $\text{SO}_2 \approx 115 \mu\text{g}/\text{cu m}$ in highest exposure Utah community versus three lower exposure towns with SO_2 in 11–36 $\mu\text{g}/\text{cu m}$ range. No TSP gradient across four communities. Effects possibly due to intermittent high SO_2 peaks.
Longitudinal study of children in southwestern US towns ⁶⁰	...	103	Significantly increased prevalence of cough among children from highest pollution area (annual average $\text{SO}_2 = 103 \mu\text{g}/\text{cu m}$; intermittent 3-hr peaks often exceeded 2,500 $\mu\text{g}/\text{cu m}$ or $\sim 1 \text{ ppm}$) in comparison to lower pollution towns (annual $\text{SO}_2 = 14 \mu\text{g}/\text{cu m}$). No TSP gradient across high- and low-pollution towns. Effects possibly due to intermittent high SO_2 peaks.

TABLE 8.
Constituents in Fresh, Undiluted Mainstream Smoke (MS)
and the Ratio in Diluted Sidestream Smoke (SS) From a
Whole Nonfilter Cigarette*

Constituent	Amount in MS	Range in SS/MS
Vapor phase		
Carbon monoxide	10–23 mg	2.5–4.7
Carbon dioxide	20–40 mg	8–11
Carbonyl sulfide	18–42 μ g	0.03–0.13
Benzene†	12–48 μ g	5–10
Toluene	100–200 μ g	5.6–8.3
Formaldehyde†	70–100 μ g	0.1–50
Acrolein	60–100 μ g	8–15
Acetone	100–250 μ g	2–5
Pyridine	16–40 μ g	6.5–20
3-methylpyridine	12–36 μ g	3–13
3-vinylpyridine	11–30 μ g	20–40
Hydrogen cyanide	400–500 μ g	0.11–0.25
Hydrazine§	32 ng	3
Ammonia	50–130 μ g	40–170
Methylamine	11.5–28.7 μ g	4.2–6.4
Dimethylamine	7.8–10 μ g	3.7–5.1
Nitrogen oxides	100–600 μ g	4–10
N-nitrosodimethylamine§	10–40 ng	20–100
N-nitrosodiethylamine§	ND–25 ng	<40
N-nitrosopyrrolidine§	6–30 ng	6–30
Formic acid	210–490 μ g	1.4–1.6
Acetic acid	330–810 μ g	1.9–3.6
Methylchloride	150–600 μ g	1.7–3.3
Particulate phase		
Particulate matter	15–40 mg	1.3–1.9
Nicotine	1–2.5 mg	2.6–3.3
Anatabine	2–20 μ g	<0.1–0.5
Phenol	60–140 μ g	1.6–3.0
Catechol	100–360 μ g	0.6–0.9
Hydroquinone	110–300 μ g	0.7–0.9
Aniline	360 ng	30
2-toluidine	160 ng	19
2-naphthylamine†	1.7 ng	30
4-aminobiphenyl†	4.6 ng	31
Benz[a]anthracene§	20–70 ng	2–4
Benz[a]pyrene*	20–40 ng	2.5–3.5
Cholesterol	22 μ g	0.9
γ -Butyrolactone§	10–22 μ g	3.6–5.0
Quinoline	0.5–2 μ g	8–11
N'-Nitrosornicotine§	200–3,000 ng	0.5–3

TABLE 8.—cont.

Constituent	Amount in MS	Range in SS/MS
NNK	100–1,000 ng	1–4
N-Nitrosodiethanolamine§	20–70 ng	1.2
Cadmium	100 ng	7.2
Nickel†	20–80 ng	13–30
Zinc	60 ng	6.7
Polonium-210†	0.04–0.1 pCi	1.0–4.0
Benzoic acid	14–28 µg	0.67–0.95
Lactic acid	63–174 µg	0.5–0.7
Glycolic acid	37–126 µg	0.6–0.95
Succinic acid	110–140 µg	0.43–0.62

*Adapted from National Research Council: The physicochemical nature of side-stream smoke and environmental tobacco smoke, in *Environmental Tobacco Smoke—Measuring Exposures and Assessing Health Effects*. Washington, DC, National Academy Press, 1986, pp 25–53.

†Human carcinogen (US Department of Health and Human Services, 1983).

‡Suspected human carcinogen (US Department of Health and Human Services, 1983).

§Animal carcinogen.⁶³

||NNK = 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone.

ber of smokers. The results of an early study in Tecumseh, Michigan (Fig 4), are typical.⁷⁵

Substantial data also establish the effects of parental smoking on children's lung functions. One statistical model derived from data on children 6 to 10 years (Fig 5) estimates that FEV₁ growth rate is reduced by 0.17% per pack of cigarettes smoked daily by the mother.⁷⁶ Although seemingly minor, the long-term effects are unknown.

Exacerbation of asthmatic symptoms by environmental tobacco smoke is an almost universal complaint. This contrasts with the inconsistency in pulmonary function changes evoked by acute experimental exposure of asthmatic adults, listed in Table 10.^{77–79} In these, experimental reductions in pulmonary function seem to relate more to preexisting dysfunction than the intensity of exposure. As noted in the National Research Council review, the frequency and extent of responsivity of asthmatics to tobacco smoke have not been defined, nor have the mechanisms for changes in airway size.⁸⁰ Local edema from irritants such as nitrogen oxides, acrolein, and ammonia; the release of substance P from capsaicin-sensitive neurons in the airway; and hypersensitivity to a tobacco glycoprotein antigen have all been incriminated. The vulnerability of children with other obstructive lung diseases, especially cystic fibrosis, is also incompletely defined.⁸⁰

TABLE 9.
Epidemiologic Studies of Early Childhood Respiratory Illnesses and Passive Smoking

Study Population	Study Design	Effect of Passive Smoking*	Comments
10,672 births in Israel, 1967–1983 ⁶⁵	Antenatal maternal smoking history, monitoring of admissions during first year of life	Significant increase in hospitalization for pneumonia and bronchitis, RR = 1.4	Dose-response relationship present; maternal smoking only
2,205 births in England, 1963–1965 ⁶⁶	Prospective cohort with annual questionnaire	Significant increase in bronchitis or pneumonia in first year of life, RR = 1.7 if one parent smoked, RR = 2.6 if both smoked	Sex of smoking parent not examined
12,068 births in Finland, 1966 ⁶⁷	Prospective cohort with follow-up of hospitalizations, physician visits, and mortality	Significant increase of hospitalization for respiratory diseases during first 5 years, RR = 1.7	Effect largest during first year; maternal smoking only and measured during pregnancy

1,265 births in New Zealand, 1977 ⁶⁸	Prospective cohort with diaries, physician and hospital record review	Significant increase in bronchitis or pneumonia during the first year of life, RR = 2.0 if mother smoked	No effect of paternal smoking; effect of maternal smoking equivocal in second year, absent in third; dose-response in first year
130 children respiratory virus infection in infancy, England ⁶⁹	Case control with 111 controls, performed 10 years after index illness	Significant effect of maternal smoking at time of illness, RR = 3.2	Effect of paternal smoking also present
1,058 births in China, 1981-70	Prospective cohort with illnesses ascertained at age 18 months by a questionnaire	Significant effect of smoke exposure at home, RR = 1.9 if > 10 cigarettes per day consumed by family	None of the mothers smoked

*RR = relative risk.

TABLE 10.
Experimental Studies of Tobacco Smoke Exposure of Asthmatic Patients

Study Population	Study Design	Effect of Passive Smoking	Comments
10 asthmatics, 16-39 yr; 10 controls, 24-53 yr ⁷⁷	1 hr at CO; 15-20 ppm	Decrease in FEV ₁ , 21.4%; FEF ₂₅₋₇₅ 19.2%; FVC, 20%; no change in controls	Patients on medications with restricted use for 4 hr; 5/10 each group claimed smoke sensitivity
14 asthmatics mean age 37 yr ⁷⁸	2 hr at CO; 24 ppm	Decrease total lung capacity ($P < 0.02$)	All on medications, 4 claimed smoke sensitivity
9 asthmatics, 19-30 yr; near normal lung function ⁷⁹	1 hr at CO; 40-50 ppm	No change expiratory flow rates; small decrease bronchial reactivity	Off medications; 6/9 claimed smoke sensitivity

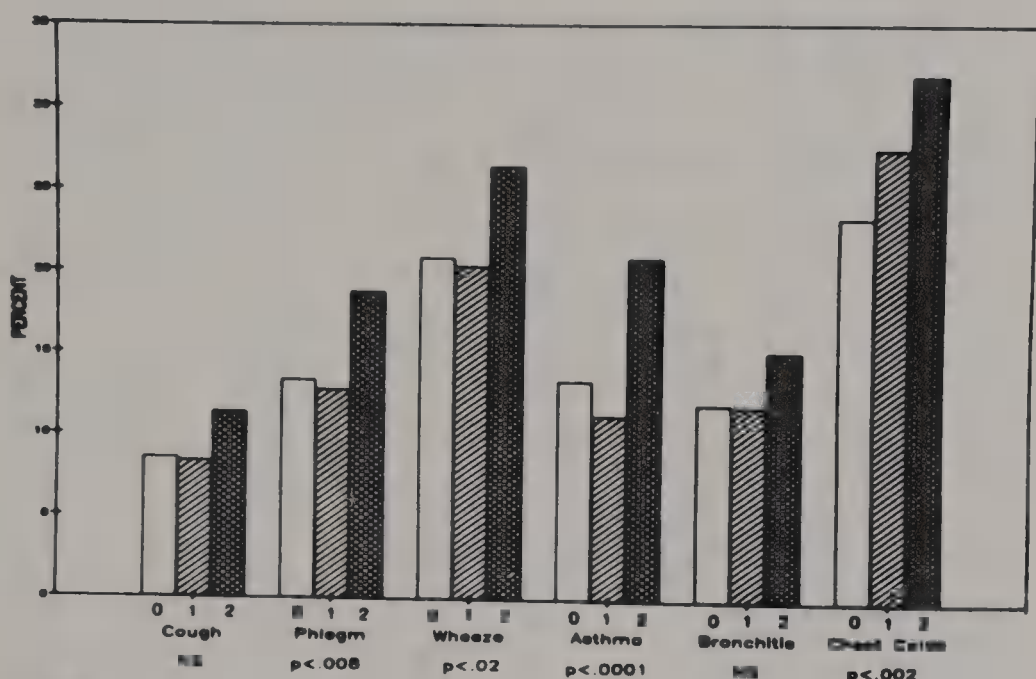
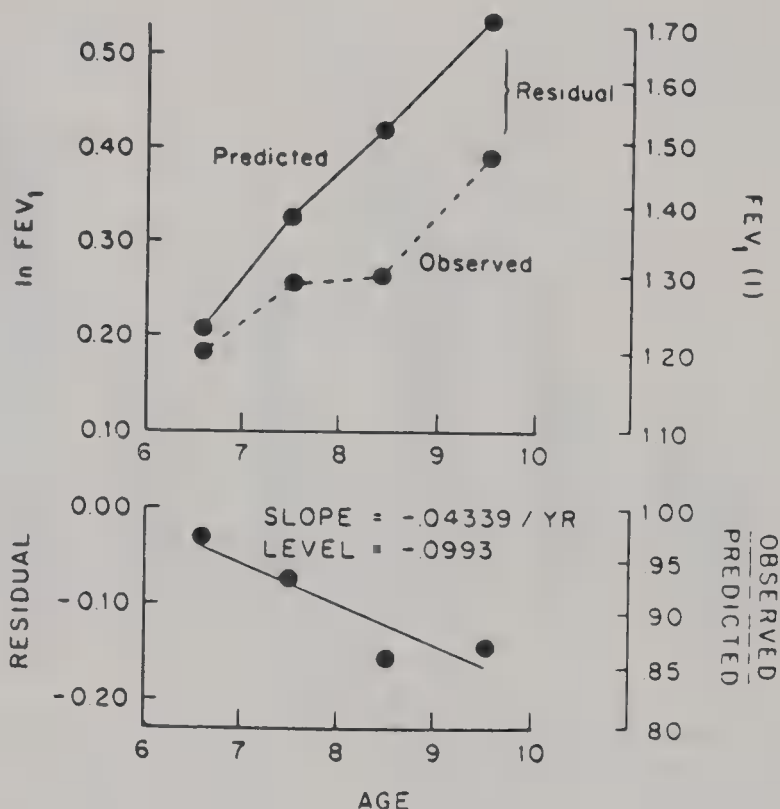


FIG 4.

Age-adjusted prevalence of respiratory conditions for males, 0 to 19 year-old non-smokers, by number of parental smokers in household, Tecumseh, 1962-1965.¹³⁶ (From Higgins M: Critical review of the relationship between passive exposure to cigarette smoke and cardiopulmonary disease, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

Household exposure to tobacco smoke is linked with increased rates of chronic ear infections and middle ear infusions in young children. Children with nasal allergies and recurrent otitis media exposed to ETS may have an increased risk for persistent ear infections.⁸¹ Convincing evidence exists that nonsmoking, pregnant women exposed to ETS on a daily basis for several hours are at increased risk for producing babies of low birth weight. There is a dose-response relationship between the number of cigarettes smoked by the father and the birth weight of children of nonsmoking, pregnant women. Studies indicating reduced growth and development in children of smokers require further differentiation of the effects of in utero exposure from subsequent childhood exposure as well as the contribution of highly suspect toxins such as CO, nicotine, and cadmium.⁸¹ The only encouraging aspect in the overwhelming evidence for the deleterious effects of environmental tobacco smoke is the significant decrease in total US consumption of cigarettes from 1980 to 1985 (Fig 6).⁷⁶

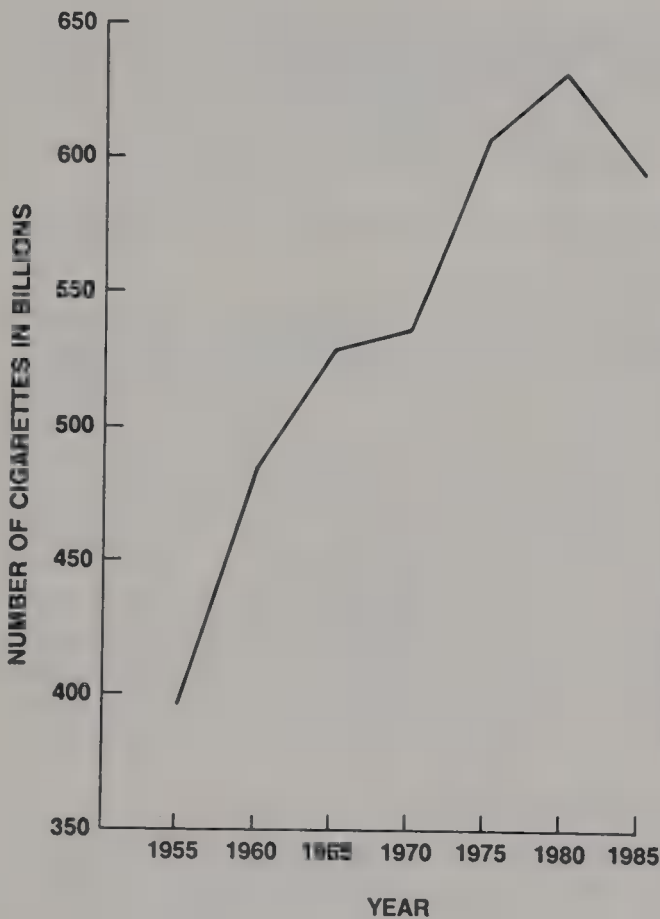
**FIG 5.**

Calculation of growth rate and level of $\ln(\text{FEV}_1)$ for an individual child. The residuals in the upper panel, i.e., the difference between observed and predicted $\ln(\text{FEV}_1)$, were regressed on age in the lower panel.⁷⁶ (From National Research Council: *Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects*, Committee on Passive Smoking, Board on Environmental Studies and Toxicology. Washington, DC, National Academy Press, 1986. Used by permission.)

Asbestos

The noncombustion fibrous particles of most concern are those of asbestos although there is increasing evidence of the fibrogenic potential of the glass fibers and mineral wools. Asbestos is the generic term for fibrous silicates of two general types: the serpentine, primarily chrysotile or "white asbestos" of more flexible texture, and the amphiboles, primarily amosite or "brown asbestos" and crocidolite or "blue asbestos." The fibers are considered equally toxic to the pulmonary epithelium and to the immune response but crocidolite and other amphiboles have the greatest carcinogenic potential for mesothelioma and bronchogenic carcinoma.⁸²

At least 90% of the asbestos used in the United States is chrysotile but the more toxic crocidolite is found in industrial installations and in the as-

**FIG 6.**

Total cigarette consumption (domestic sales), 1955–1985. (From National Research Council: *Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects*, Committee on Passive Smoking, Board on Environmental Studies and Toxicology. Washington, DC, National Academy Press, 1986. Used by permission.)

bestos-cement pipe used to transport drinking water. Tremolite asbestosis, another amphibole, has recently been identified in playbox sand.⁸³

Asbestos is a prime example of an environmental risk magnified by the universal exposure to low levels beginning at a young age. The risk model derived from the observed incidence of mesothelioma in insulation workers suggests an increase that is exponentially related to the years since the first exposure or time raised to a power of 3.2.⁸⁴ If the risk is exponentially related to time although only linearly related to dose, extremely low-level exposure of a young child may have a higher risk than occupational exposure of an adult. Although measurements of the mass of asbestos per cubic meter of air are often meaningless since counts by optical microscopy include many types of nonasbestos fibers, the concentrations in public buildings as determined by transmission electron microscopy rarely exceed 0.0005 fibers/ml. The mesothelioma model predicts that 20 years of exposure to 0.0005 fibers/ml for 40 hours per week has a lifetime risk of one case/100,000. When projected to exposure of the entire US population of 220,000,000, this model predicts 2,200 excess cases of mesothelioma even at minimal levels of exposure. Of respiratory cancers associated

with asbestos, pleural mesothelioma has the shortest latent period and has been reported in a small number of children with exposure to ambient dust from mining.⁸⁵ A pathogenetic mechanism in addition to asbestos has been proposed for these cases⁸⁶ and the data required for a reliable prediction of the risk of childhood exposure are seriously inadequate.

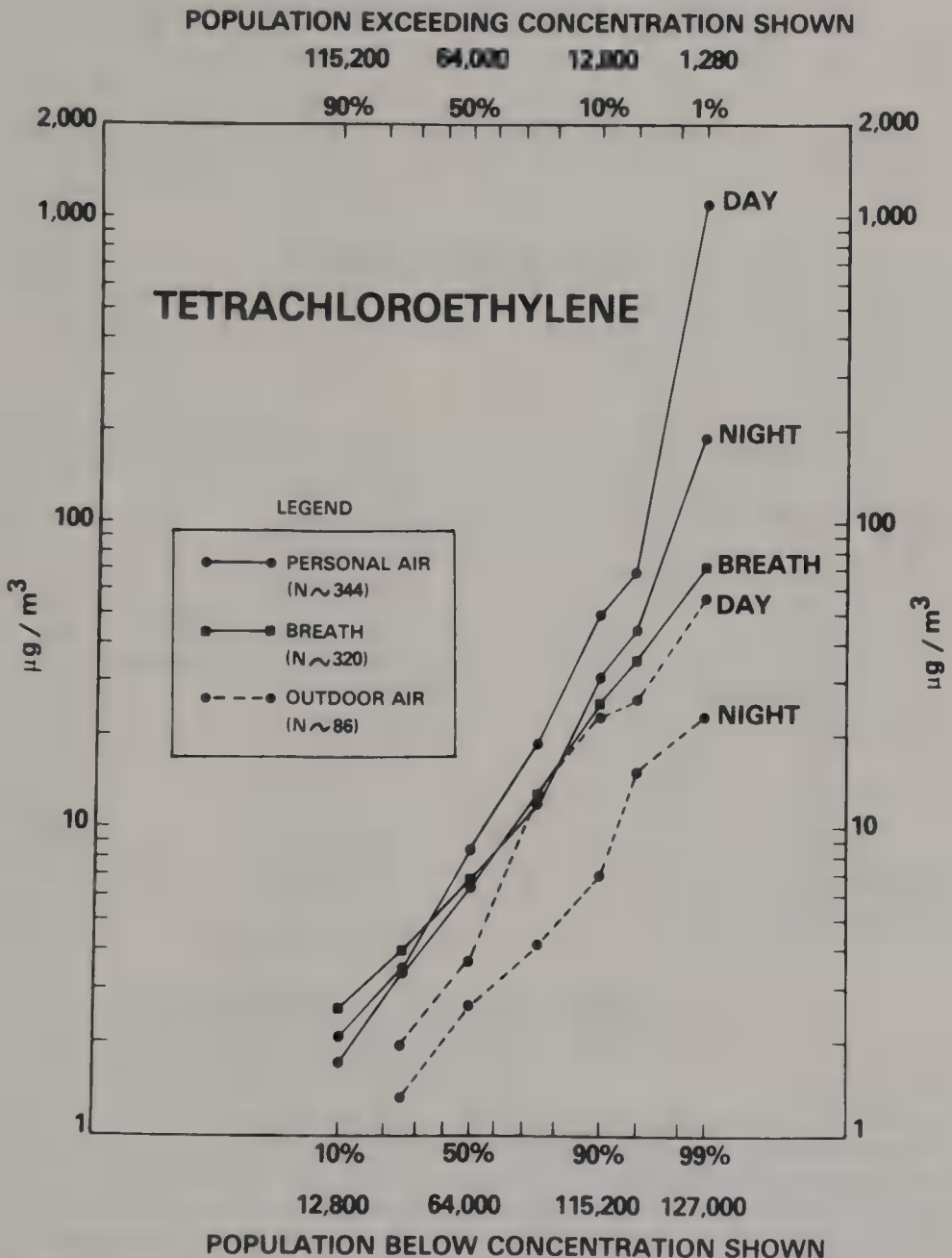
Volatile Organic Compounds

Increasing emphasis is placed on over 350 volatile organic compounds (VOCs) that have been identified in concentrations over 0.001 ppm in indoor air.^{63, 87} Organic compounds are emitted by almost all materials and products used in construction, furnishings, consumer products and pesticides. A large variety are produced from combustion fuels, tap water, and tobacco smoke and by human metabolism. Examples of organic compounds and their sources are shown in Table 11.^{81, 87} The aliphatic hydrocarbons include the alkanes, alkenes, and alkynes from fuels, aerosol propellants, and solvents. They also include the organic compounds considered primary contributors to indoor odors: limonene, α -pinene, *n*-hexanol, 1,3-xylene, and other terpenes. Halogenated hydrocarbons are typically present in tap water, household air, and human breath. Tetrachloroethylene consistently shows the highest breath air correlations of any chemical in this group.

The Total Exposures Assessment Methodology (TEAM) study by the Environmental Protection Agency (EPA) determined 12-hour integrated exposures and corresponding breath levels of the 20 to 25 most common VOCs in 650 households in six cities.^{88, 89} Data from 350 participants in Bayonne and Elizabeth, New Jersey, were used to estimate the percent of population exposed to progressively higher concentrations. Tetrachloroethylene in personal air monitors indoors exceeded 670 $\mu\text{g}/\text{cu m}$ in 1% of the participants; this is almost three orders of magnitude below the OSHA permissible exposure limit, the time weighted average for a 40-hour week, of 335 $\text{mg}/\text{cu m}$ (Fig 7). A similar indoor/outdoor ratio is evident in the data projecting exposure to chloroform (Fig 8). The higher nighttime exposures of chloroform as measured by personal air monitors in the household reflect the high concentrations released from chlorinated tap water and absorbed by both the skin and the lungs.

Persistence of the chlorinated hydrocarbon pesticides in the household, particularly chlordane, is frequently associated with illness⁹⁰ and resulted in removal of chlordane-heptachlor termite treatments from the market. The air levels of the most common residential pesticides as reported in three recent studies are summarized in Table 12.⁹¹⁻⁹³

Aliphatic hydrocarbons, including carcinogenic compounds such as benzene and styrene, are ubiquitous. Benzene is now banned from household products but is present in gasoline at concentrations up to 15%. Styrene is emitted by the majority of plastics and insulation.

**FIG 7.**

Tetrachloroethylene: Estimated frequency distributions of personal air exposures, outdoor air concentrations, and exhaled breath values for the combined Elizabeth-Bayonne target population of 128,000.^{88,89} The maximal time weighted average (TWA) for occupational exposure to tetrachloroethylene (perchloroethylene) is 335,000 µg/cu m. (From Wallace LA, Pellizzari ED, Gordon SM: Organic chemicals in indoor air: A review of human exposure studies and indoor air quality studies, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

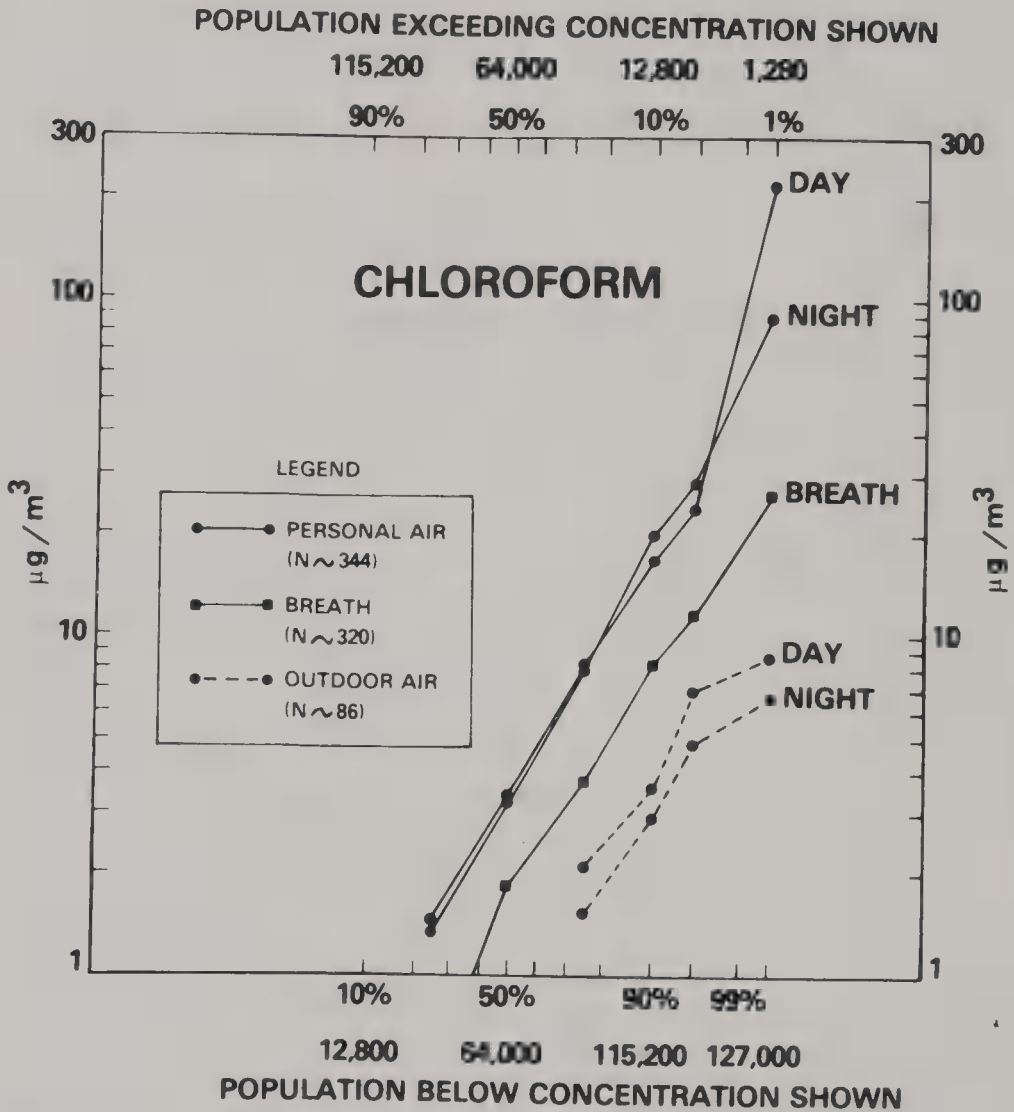


FIG 8.

Chloroform: Estimated frequency distributions of personal air exposures, outdoor air concentrations, and exhaled breath values for the combined Elizabeth-Bayonne target population of 128,000.^{88,89} The occupational TWA for chloroform is 50,000 µg/cu m. (From Wallace LA, Pellizzari ED, Gordon SM: Organic chemicals in indoor air: A review of human exposure studies and indoor air quality studies, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

Miscellaneous chemicals include methyldiisocyanate and toluene diisocyanate, released from polyurethane foam insulation and weather proofing. Both are incriminated as causes of allergic symptoms as are the emissions from the epoxy resins including trimellitic acid, phthalic acid, anhydride, and triethylene tetramine.

TABLE 11.
Examples of Organic Compound Types and Potential Indoor
Sources^{81, 87}

Pollutant Type	Example	Indoor Sources
Aliphatic hydrocarbons	Propane, butane, hexane, limonene	Cooking and heating fuels, aerosol propellants, cleaning compounds, refrigerants, lubricants, flavoring agents, perfume base
Halogenated hydrocarbons	Trichlorethylene, tetrachloroethylene, 1,1,1-trichloromethane, chloroform, methylene chloride, PCBs, benzyl and benzal chloride	Aerosol propellants, fumigants, pesticides, refrigerants, and degreasing, dewaxing, and dry cleaning solvents, tap water, appliances, transformers, plasticized vinyl tile
Aromatic hydrocarbons	Benzene, toluene, xylenes, styrene	Paints, varnishes, glues, cleaners, petroleum products
Alcohols	Ethanol, methanol	Window cleaners, paint thinners, cosmetics, adhesives, human breath
Ketones	Acetone	Lacquers, varnishes, polish removers, adhesives
Aldehydes	Formaldehyde, acetaldehyde	Fungicides, germicides, disinfectants, textiles, paper, cardboard, particle board, cosmetics, flavoring agents, etc.
Miscellaneous	Toluene diisocyanate Phthalic acid anhydride, trimellitic acid, triethylene tetramine Sodium dodecyl sulfate	Polyurethane foam aerosols Epoxy resins Carpet shampoo

Formaldehyde is the most intensively studied of the aldehyde compounds associated with health effects and has been officially classified as carcinogenic. The thresholds for eye irritation and detectable neurophysiologic responses are summarized in Table 13.⁹⁴ All of the clinical studies involve an undetermined degree of concurrent emissions of resins, glues, and plastics. Asthma is considered a relatively uncommon manifestation of formaldehyde sensitivity but has been established with some frequency in our clinic. It is suspected in children with respiratory symptoms developing

TABLE 12.
Measurements of Pesticides in
Residences

Pesticide	Range, µg/cu m
Chlordane ⁹¹	0.1–10.0
Ronnel ^{91–93}	.01–20.0
Dursban ⁹¹	0.2–2.0
Dichlorvos (DDVP) ^{91, 92}	0.05–28.0
Malathion ^{91–93}	0.1–2.0
Diazinon ^{91, 92}	0.01–2.0

TABLE 13.
Acute Human Health Effects of Formaldehyde at Various
Concentrations*

Formaldehyde Concentration (ppm)	Reported Effects
0.0–0.5	None reported
0.05–1.5	Neurophysiologic effects (optical chronaxy, sensitivity of dark-adapted eyes)
0.05–1.0	Odor threshold
0.01–2.0	Eye irritation (other pollutants present)
0.10–25	Upper airway irritation
5–30	Lower airway and pulmonary effects
50–100	Pulmonary edema, inflammation, pneumonia
>100	Death

*Adapted from National Research Council: *Formaldehyde and Other Aldehydes*. Washington, DC, National Academy Press, 1981.

in a new or remodeled residence or who live in a mobile home, which is typically both airtight and almost completely lined with particle board.

The VOCs are most often incriminated in the sick building syndrome, characterized by varying combinations of the symptoms listed in Table 14.⁹⁵ Since these symptoms are readily perpetuated and enhanced by psychosocial feedback, investigation of a public epidemic should be handled by experienced epidemiologists and industrial hygienists. Parents who sus-

pect that symptoms relate to their own home soon find that commercial testing is inordinately expensive. The local health department may help narrow the search to molds, pesticides, or VOCs but greater selectivity is required before testing. If a VOC is suspect, one approach is a passive organic vapor monitor that will analyze a maximum of three compounds from a list of over 90.⁹⁶ The emission rates of organic vapors usually decline with time unless they result from weathering of the linings of air ducts. Formaldehyde emissions are typically below 0.2 ppm after 2 years of occupancy.

Ventilation is not always the simple answer to the sick building syndrome since an increase in air flow often increases the rate of emission. As shown in Table 15⁹⁷ an increase from 0.54 to 2.71 air exchanges per hour

TABLE 14.
The Sick Building Syndrome⁹⁵

Irritation of eyes, nose, and throat
Dry mucous membranes and skin
Skin rashes
Mental fatigue, dizziness
Airway infections, cough
Hoarseness, wheezing
Nonspecific hypersensitivity reactions
Nausea, diarrhea

TABLE 15.
Emission Rates From Particle Board ($\mu\text{g/sq m/hr}$)⁹⁷

Air Exchange (hr^{-1})	Loading* (sq m/cu m)	Formaldehyde	Acetone	Hexanal	Others‡
2.71	1.96	154	37	15	27
0.54	1.17	95	41	26	26
3.61	0.78	230	38	20	31
0.54	0.39	140	37	24	27

*Sq m board per cu m air.
‡The sum of emission rates for propanol, butanone, benzaldehyde, and benzene.

increases the emission of formaldehyde from particle board.⁹⁷ Emissions are also inversely related to the square meters of particle board per cubic meter of air or loading factor. Treatment with ammonia, heat, and time have all been effective in formaldehyde abatement but surface sealing or removal of the source may be the only answer for other volatile organic chemicals.

Radon and Nonionizing Radiation

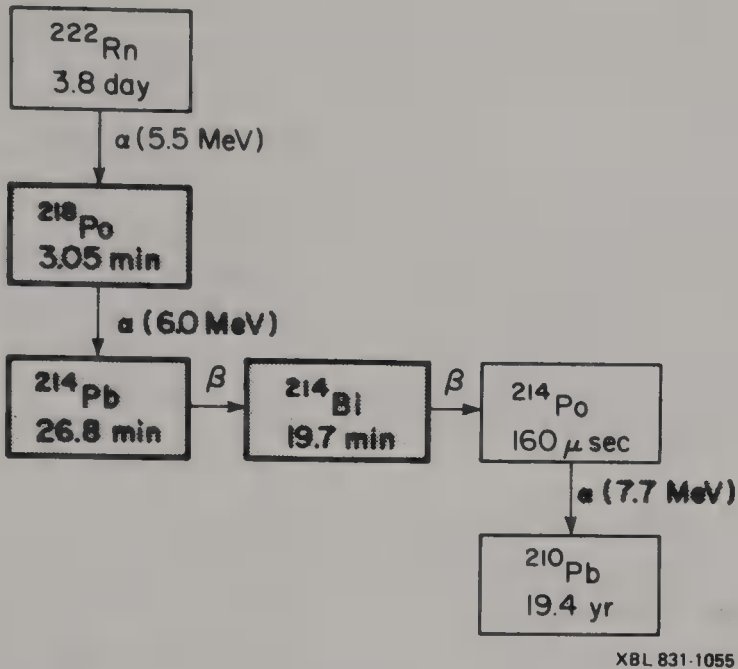
Radon

Although the significance of low-level naturally occurring radiation in human carcinogenesis is still to be defined,⁹⁸ radon 222 has emerged as a prime compound for risk assessment for three reasons: (1) the inhaled daughters of radon 222 account for approximately half of our baseline radiation; (2) integrated measurements of radon 222 are an easily available index of its daughter isotopes; and (3) there are human epidemiologic data from uranium miners defining the multiplicative interaction of α -radiation with smoking. In the field of risk assessment, these rank as hard data.

Radon 222 arises from trace concentrations of radium 226 in the earth's crust. As illustrated in Figure 9, the most chemically and radiologically active daughters, polonium 218 and polonium 214, are short-lived α -emitters that decay in the tracheobronchial tree during respiration of the fine particles.⁹⁹ These progeny also agglomerate with particles and droplets and fix to surfaces, confounding the seemingly direct relation of air concentration to respiratory dose. Given the relatively short half lives of the polonium isotopes, their concentration must be in equilibrium with a sustained source of radon 222. This permits integrated radon measurements to reflect the probable α -radiation of the lung. It is generally accepted that infiltration of gas from the subsoil is the primary source of radon in US homes; under some circumstances, building materials or water supplies from deep wells or ground water with little prior aeration make a substantial contribution.

The median US residential concentration of radon is considered to be 1.5 pCi/L but concentrations of one or two orders of magnitude (10 to 100 pCi/L) are found in some homes. The current EPA action level is 4 pCi/L. Legislation is pending to permit abatement procedures to qualify as tax-deductible medical expenses. The EPA Office of Radiation Programs has certified over 300 private radon testing firms. Passive exposure of a charcoal canister for 2 to 7 days is usually employed; more accurate techniques require 1 or more months.¹⁰⁰

Since the carcinogenic implications of residential radon derive from the chronic high exposure of uranium miners, the validity of extrapolation to low-dose exposure is uncertain, as is the effect of age at exposure. Two

**FIG 9.**

Decay chain and half lives of radon 222 and its daughters. The darkly outlined isotopes have the maximal potential for retention in the lung and acute irradiation by alpha decays (dark print).⁹⁹ (From Nero AV Jr: Indoor concentrations of radon 222 and its daughters: Sources, range, and environmental influences, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985. Used by permission.)

divergent estimates of the significance of the median household exposure are given in Table 16.¹⁰¹⁻¹⁰³ One current risk assessment model estimates that background exposure to radon progeny at 0.5 pCi/L accounts for approximately 10% of lung cancer in nonsmokers or 30% at 1.5 pCi/L if linearity of the dose response is assumed.¹⁰² This estimate supports the current consensus that the risk of lifetime exposure to residential levels of 1.5 pCi/L is equivalent to the lung cancer risk for nonsmokers attributed to environmental tobacco smoke. Wilson and Crouch¹⁰³ calculate that 1.5 pCi/L is equated with 500 mrem/yr and although this is to the lungs, not the whole body, they predict 13,500 excess cancers per year. The current EPA estimate is that the aggregate effect of all radon exposures is 5,000 to 20,000 lung cancer deaths per year, the lower bound presumably representing risk for nonsmokers.

Despite the lack of epidemiologic data to define risk, a large number of options have been proposed and implemented to reduce indoor radon. These include forced ventilation and pressurization of the house, foundation sealants, and various modes of ventilation of the subslab and underlying soil.⁸¹

TABLE 16.
Annual Lung Cancer Deaths in the United States

Total, 1987	136,000
Nonsmokers, total, 1985 ¹⁰¹	12,000 (100%)
Spouse of smoker ¹⁰¹	2,440–5,160 (20%–42%)
Radon 1.5 pCi/L ¹⁰²	3,660 (30%)
Estimated excess cancers, US population ¹⁰³	
Radon, 1.5 pCi/L (500 mrem/yr)	13,500
Medical x-rays	1,100

Nonionizing Radiation

In common with radon, the health risk of electric and magnetic fields generated by high-voltage power lines and by indoor appliances has been legally recognized as a litogen before scientific definition as a teratogen or carcinogen. In a 1985 case in Harris County, Texas, a jury found a reasonable hazard to the health of 4,000 children in schools near a 345-kV transmission line.¹⁰⁴ The evidence for this relates to the immunologic and teratogenic effects of high-voltage exposure for animal systems and human cell cultures^{105–110} and to two epidemiologic studies: correlation of an increased incidence of childhood cancers, all types, with power distribution lines in front of homes¹¹¹ and an increase in nervous system tumors in children exposed to 50-Hz fields from power lines.¹¹² A published correlation of the use of an electric blanket or electrically heated waterbed with an increase in first trimester abortions, reduced birth weight, and prolonged gestation is provocative for the lack of discrimination from multiple other factors associated with use of these household appliances.¹¹³

Pathogens and Allergens

Although most infections are spread by direct contact, the high percentage of immunocompromised children and adults in any hospital population necessitates consideration of infections due to airborne contamination. One of the earliest and best documented of these was an outbreak of tuberculosis aboard the submarine USS Richard Byrd in which a tuberculin reactivity developed in 140 of 308 crew after 6 months inhaling recycled air contaminated with infected droplets from a seaman with a cavitary lesion.¹¹⁴ Airborne outbreaks of smallpox, varicella, measles, rubella, and anthrax have all occurred.^{114, 115} Saprophytic fungi such as *Phialophora*,

Fusarium, *Sporothrix*, and *Geotrichum* have all been implicated in operating room contamination by vaporizers. The most common clinical infections spread by airborne water droplets are summarized in Table 17.¹¹⁶⁻¹²⁶

Mycointoxications

Fungi that produce mycotoxins may be pathogenic, allergenic, and also toxicogenic. The best known of the mycotoxins are produced by three genera: *Aspergillus*, *Penicillium*, and *Fusarium*. *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus parasiticus* produce aflatoxins and tremorgenic mycotoxins although others such as *Aspergillus niger* are not toxicogenic. At least three of the aflatoxins, B₁, B₂, and G₁, are potent liver and systemic toxins, as well as animal carcinogens and cellular mutagens.¹²⁷

Trichothecene mycotoxins are produced by a variety of molds including *Stachybotrys*, *Fusarium*, *Trichothecium*, *Trichoderma*, *Aeromonium*, *Cylindocarpon* and *Myrothecium*. Low concentrations of the trichocenes produce immunosuppression, hematosuppression, gastrointestinal lesions, and reproductive and neurologic toxicity plus symptoms overlapping those of the stuffy building syndrome.

An environmental horror story, rapidly retrieved from the press by "stuffy building" occupants with high levels of anxiety, is the report¹²⁸ of a familial outbreak of *Stachybotrys* toxicosis in Chicago due to heavy contamination by *Stachybotrys atra* of Celotex fiberboard and of a cool air return duct. Symptoms included muscle aches and pains, sore throat, headache, fatigue, diarrhea, dermatitis, focal alopecia, malaise, and severe depression with suicide by one son. The mycotoxins verrucarins B and trichoverins A and B were isolated from the fungus samples from the ceiling board and air duct. The symptoms did not recur after thorough cleaning and removal of the contaminating fungi. Bacterial and amoebic endotoxins, detected in quantitative air samples by the limulus amoebocyte lysate test, may also have a direct toxic effect or elicit sensitization as defined for humidifier fever.

Allergens

Any increase in the severity of allergic rhinitis or asthma when indoors is evidence for the accumulation of biologically active aerosols: human and animal skin scales, plant fibers, bacteria and dermatophytes shed from skin, and cockroach, mite, and arachnid fragments and feces. These may be intrinsically allergenic or support the growth of proximate allergens, primarily molds and house mites.

The house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are thought to cause more symptomatic days than any other single allergen. Since reproduction of mites is favored at indoor air temperatures of 23 C and a relative humidity over 55%, maintaining the relative humidity below 50% is considered the most important factor in re-

TABLE 17.
Airborne Infections

Organism	Transmission	Disease
<i>Aspergillus</i> sp	Pollution of air intake from old construction site, ¹¹⁶ from helicopter rotor draft, reverse function of air fan ¹¹⁷	Aspergillosis in immunosuppressed patients
<i>Legionella</i> sp	Contaminated cooling towers and evaporative condensers, ^{118, 119} air conditioners, ¹²⁰ showers, faucets, and vacuum breakers ¹²¹⁻¹²³	Legionnaire's disease and Pontiac fever
Thermophilic actinomycetes, ¹²⁴ <i>Bacillus subtilis</i> , ¹²⁵ <i>Naegleria</i> sp ¹²⁴	Contaminated humidifiers and air conditioners	Humidifier fever (allergic alveolitis): fever, malaise, arthralgia, headache, dyspnea with interstitial infiltrate 4-12 hr after exposure; reproduced by inhalation of aqueous extract of antigen ¹²⁶

ducing mite numbers. The active antigens of house mites are freely soluble glycoproteins of 24,000-dalton molecular weight. These persist, although in decreasing numbers, after treatment by acaricides or dehydration, accounting for perennial symptoms. Although quantitation of house mite allergens is difficult, they are additional examples of indoor air pollutants that actually increase with forced ventilation. Abatement involves the time-honored techniques of encasement and removal.

Sensitization to mold spores is thought to require prolonged or repeated colonization of the airway, possible for molds with favorable aerodynamic characteristics and active growth at 37 C. These are primarily species of *Aspergillus*, *Candida*, *Scedosporium*, *Scopulariopsis*, *Geotrichium*, *Penicillium*, and *Paecilomyces*.¹²⁹

Investigation of mold pollution is indicated by the history, skin testing, and antibody testing of the patient serum. The enumeration and quantitation of molds are complex, and the methodologies are summarized in Table 18.^{114, 115, 129} Sampling should be conducted in areas where water typically collects: bathrooms, basements, refrigerator pans, undersink cabinets, clothes dryer ducts, humidifiers, and cool air return ducts. Settle plates and agar slides are commonly used to indicate the presence of molds but do not adequately represent small-sized spores, slow growers, and temperature-sensitive spores that include those producing trichocene

TABLE 18.
Microbial Sampling^{114, 115, 129}

Method	Disadvantage
Collection	
Settle plate/Agar slide	Overrepresents large particles but useful for screening
Impaction sampler (Rotorod)	Inefficient < 15 μ (<i>Cladosporium</i> , <i>Aspergillus</i>)
Volumetric pump (Andersen) } Suction trap: 7 day (Burkard) }	Preferred methods
Analysis	
Visual count	Poor differentiation
Viable particle culture	Underestimates slow growers; viability irrelevant to hypersensitivity
Antigen specific (ELISA)	Tests only specific antigens; cross reactivity common ¹³²
Diffusion gel using patient's or synthesized antibodies	Excess false positives, negatives ¹³² , no detection mycotoxins

mycotoxins. As in most instances of suspected airborne pollutants, testing is more costly than a detailed evaluation of potential sources and thorough cleaning.

Abatement

The incorporation of continuous whole-wall plastic sheeting in new and renovated houses is considered the major factor in reducing residential infiltration to 0.5 air changes per hour, the lower limit of acceptable ventilation. Measures to increase air exchange such as air-to-air heat exchangers are not widely used. Increased ventilation, despite the increased energy cost, is the simplest mode of reducing combustion gases, particulates, fibers, tobacco smoke, and many volatile organic gases.

Of the various air cleaning devices electrostatic precipitators and high-efficiency particulate air filters have an efficiency beyond that contributed by the increase in air flow. They have a significant short-term benefit on contamination by particulates including allergens but require scrupulous maintenance for sustained efficiency.^{130, 131}

Increased ventilation may paradoxically increase the rate of emissions from static surfaces such as the combinations of styrene, epoxy resins, phenol, and formaldehyde from pressed board; benzyl and benzal chlorides from plasticized vinyl tile; and sodium dodecyl sulfate detergent and mite allergens from carpets and bedding. Removal, encasement, or other sealing-off of the source is required. Mold growth is rarely evident on visible surfaces but cleaning, sodium hypochlorite, ultraviolet treatment, and absolute dehydration of high-humidity sites are effective. Persistent contamination of the subsoil and wooden footings by chlordane termite treatment and other pesticides requires sealing with concrete or plastic. Microbial degradation of polychlorinated biphenyls (PCBs) and other residual halogenated hydrocarbons, a crucial need, is still under development.

A whole new subsidized industry has developed to reduce the gaseous entry of soil radon. Since air pressurization is energy expensive in cold climates, most of these involve tight sealing of the basement or ventilation of the crawl space plus soil ventilation by drain tile systems as well as fans in the soil drains or in the hollow block walls of the basement.

Summary

A major contribution of the pediatrician is to help families rank the multitude of pollutants according to their known risk for child health. Elimination of household smoking and completely effective venting of indoor heating devices are beneficial to all and mandatory in homes of allergic children. Acute releases of NO₂ by gas ranges and ovens may be a significant factor in an increased incidence of respiratory infection, especially in children under two years. Despite intensive investigation, immunosuppres-

sive and other health effects have not been defined for indoor levels of PBBs, PCBs, and related halogenated hydrocarbons. The analytic ability to determine nanomolar concentrations of numerous toxic chemicals opens a Pandora's box of inquiry. New methods, particularly immunologic, are urgently needed to quantitate the dose response to multiple combinations of chemicals and determine their significance for the health of the "tight-box" generation of children.

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References

1. Tredgold T: *The Principles of Warming and Ventilation: Public Buildings*. London, England, J Taylor Publisher, 1824.
2. Carnelley T, Haldane JS, Anderson AM: The carbonic acid, organic matter, and microorganisms in air, more especially of dwellings and schools. *Philos Trans B* 1887; 178:61-111.
3. Billings JS: *Ventilation and Heating*. New York, Engineering Record, 1893, pp 120-130.
4. *Comparison of Indoor and Outdoor Air Quality*, EPRI EA-1733, RP 1309. Palo Alto, Calif, Electrical Power Research Institute, 1984.
5. *Trends in Television: 1950 to Date*. Research Department, Television Bureau of Advertising, Inc, March 1984, p 2.
6. Robertson AS, Burge PS, Hedge A, et al: Comparison of health problems related to work and environmental measurements in two office buildings with different ventilation systems. *Br Med J* 1985; 291:373-376.
7. U.S. Department of Energy: *Indoor Air Quality Environmental Information Handbook*, DOE/EV/10450-1. US Dept of Energy, 1985.
8. Akland GG, Hartwell TD, Johnson TR, et al: Measuring human exposure to carbon monoxide in Washington, DC, and Denver, Colorado, during the winter of 1982-1983. *Environ Sci Technol* 1985; 19:911-918.
9. US Environmental Protection Agency: *Revised Evaluation of Health Effects Associated with Carbon Monoxide Exposure: An Addendum to the 1979 Air Quality Criteria Document for Carbon Monoxide*, EPA report no. EPA-600/8-83-033F. Springfield, Va, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, NTIS, PB85-103471, 1984.
10. Horvath SM, Raven PB, Dahms TE, et al: Maximal aerobic capacity at different levels of carboxyhemoglobin. *J Appl Physiol* 1975; 38:300-303.
11. Drinkwater BL, Raven PB, Horvath WM, et al: Air pollution, exercise and heat stress. *Arch Environ Health* 1974; 28:177-181.
12. Anderson EW, Andelman RJ, Strauch JM, et al: Effect of low-level carbon monoxide exposure on onset and duration of angina pectoris: A study on 10 patients with ischemic heart disease. *Ann Intern Med* 1973; 79:46-50.

13. Klein JP, Forster HV, Stewart RD, et al: Hemoglobin affinity for oxygen during short-term exhaustive exercise. *J Appl Physiol* 1980; 48:236-242.
14. Stewart RD, Newton PE, Barretta ED, et al: Physiological response to aerosol propellants. *Environ Health Perspect* 1978; 26:275-285.
15. Weiser PC, Morrill CG, Dickey DW, et al: Effects of low-level carbon monoxide exposure on the adaptation of healthy young men to aerobic work at an altitude of 1,610 meters, in Folinsbee LJ, Wagner JA, Borgia JF, et al (eds): *Environmental Stress: Individual Human Adaptations*. New York, Academic Press, Inc, 1978, pp 101-110.
16. Putz VR, Johnson BL, Setzer JV: *Effects of CO on Vigilance Performance. Effects of low level carbon monoxide on divided attention, pitch discrimination, and the auditory evoked potential*, DHEW (NIOSH) publication no. 77-124. Cincinnati, OH, US Dept of Health, Education, and Welfare, National Institute for Occupational Safety and Health, 1976.
17. Bender W, Gothert M, Malorny G, et al: Effects of low carbon monoxide concentrations in man. *Arch Toxicol* 1971; 27:142-158.
18. Schulte JH: Effects of mild carbon monoxide intoxication. *Arch Environ Health* 1973; 5:524-530.
19. O'Donnell RD, Mikulka P, Heinig P, et al: Low level carbon monoxide exposure and human psychomotor performance. *Toxicol Appl Pharmacol* 1971; 18:593-602.
20. McFarland RA, Roughton FJW, Halperin MH, et al: The effects of carbon monoxide and altitude on visual thresholds. *J Aviat Med* 1944; 15:381-394.
21. McFarland RA: Low level exposure to carbon monoxide and driving performance. *Arch Environ Health* 1973; 27:355-359.
22. Salvatore S: Performance decrement caused by mild carbon monoxide levels on two visual functions. *J Saf Res* 1974; 6:131-134.
23. Wright G, Randell P, Shephard RJ: Carbon monoxide and driving skills. *Arch Environ Health* 1973; 27:349-354.
24. Rummo N, Sarlanis K: The effect of carbon monoxide on several measures of vigilance in a simulated driving task. *J Saf Res* 1974; 6:126-130.
25. Putz VR, Johnson BL, Setzer JV: A comparative study of the effects of carbon monoxide and methylene chloride on human performance, in *Proceedings of the 1st Annual NIOSH Science Symposium*. Chicago, Ill, Pathotox Publishing Co, 1979.
26. Putz VR: The effects of carbon monoxide on dual-task performance. *Human Factors* 1979; 21:13-24.
27. Haider M, Groll-Knapp E, Hoeller H, et al: Effects of moderate CO dose on the central nervous system: Electrophysiological and behavior data and clinical relevance, in Finkel AJ, Duel WC (eds): *Clinical Implications of Air Pollution Research, Air Pollution Medical Research Conference*. American Medical Association, December 1974, San Francisco, CA. Acton, Mass, Publishing Sciences Group, Inc, 1976, pp 217-232.
28. Winneke G: Behavioral effects of methylene chloride and carbon monoxide as assessed by sensory and psychomotor performance, in Xintaras C, Johnson BL, de Groot I (eds): *Behavioral Toxicology: Early Detection of Occupational Hazards*. Proceedings of a workshop, June, Cincinnati, OH. US Dept of Health, Education, and Welfare, (NIOSH), 1973, pp 75-126.
29. Christensen CL, Gliner JA, Horvath SM, et al: Effects of three kinds of hypoxias on vigilance performance. *Av Sp Env Med* 1977; 48:491-496.

30. Benignus VA, Otto DA, Prah JD, et al: Lack of effects of carbon monoxide on human vigilance. *Percept Mot Skills* 1977; 45:1007-1014.
31. Fechter LD, Mactutus CF, Storm JE: Carbon monoxide and brain development. *NeuroToxicology* 1986; 7:463-474.
32. US Environmental Protection Agency; *Air Quality Criteria for Oxides of Nitrogen: Final Report*, EPA Report No. EPA-600/8-82-026F. Research Triangle Park, NC, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, NTIS, Springfield, VA, PB83-16333, 1982.
33. World Health Organization: Acrylonitrile, in *Air Quality Guidelines*. Geneva, Switzerland, World Health Organization, 1987, vol 1, pp 1-1-1-23.
34. Ahmed T, Marchette B, Danta I, et al: Effect of 0.1 ppm NO₂ on bronchial reactivity in normals and subjects with bronchial asthma. *Am Rev Respir Dis* 1982; 125:152.
35. Ahmed T, Danta I, Dougherty RL, et al: Effect of NO₂ (0.1 ppm) on specific bronchial reactivity to ragweed antigen in subjects with allergic asthma. *Am Rev Respir Dis* 1983; 127:160.
36. Hazucha MJ, Ginsberg JF, McDonnell WF, et al: Effects of 0.1 ppm nitrogen dioxide on airways of normal and asthmatic subjects. *J Appl Physiol Respir Environ Exercise Physiol* 1983; 54:730-739.
37. Kagawa J, Tsura K: Respiratory effects of 2-hour exposure to ozone and nitrogen dioxide alone and in combination in normal subjects performing intermittent exercise. *Nippon Kyobu Shikkan Gakkai Zasshi* 1979; 17:765-774.
38. Kleinman MT, Bailey RM, Linn WS, et al: Effects of 0.2 ppm nitrogen dioxide on pulmonary function and response to bronchoprovocation in asthmatics. *J Toxicol Environ Health* 1983; 12:815-826.
39. Bauer MA, Utell MJ, Morrow PE, et al: 0.30 ppm nitrogen dioxide inhalation potentiates exercise-induced bronchospasm in asthmatics. *Am Rev Respir Dis* 1984; 129:A151.
40. Rogers SA: Indoor air quality and environmentally induced illness, in *Proceedings of the ASHRAE Conference: Managing Indoor Air for Health and Energy Conservation*, IAQ 1986, April, Atlanta, American Society of Heating, Refrigerating, and Air-Conditioning Engineers Inc, 1986, pp 71-77.
41. Speizer FE, Ferris BJ Jr, Bishop YMM, et al: Respiratory disease rates and pulmonary function in children associated with NO₂ exposure. *Am Rev Respir Dis* 1980; 121:3-10.
42. Hasselblad V, Humble CG, Graham MG, et al: Indoor environmental determinants of lung function in children. *Am Rev Respir Dis* 1981; 123:479-485.
43. Ware JH, Dockery DW, Spiro A III, et al: Passive smoking, gas cooking and respiratory health of children living in six cities. *Am Rev Respir Dis* 1984; 129:366-374.
44. Florey CduV, Melia RJW, Chinn S, et al: The relation between respiratory illness in primary school children and the use of gas for cooking: III. Nitrogen dioxide, respiratory illness and lung infection. *Int J Epidemiol* 1979; 8:347-353.
45. Keller MD, Lanese RR, Mitchell RI, et al: Respiratory illness in households using gas and electricity for cooking: I. Survey of incidence. *Environ Res* 1979; 19:495-503.
46. Keller MD, Lanese RR, Mitchell RI, et al: Respiratory illness in households

- using gas and electricity for cooking: II. Symptoms and objective findings. *Environ Res* 1979; 19:504-515.
47. Dodge R: The effects of indoor pollution on Arizona children. *Arch Environ Health* 1982; 37:151-155.
 48. Ekwo EE, Weinberger MM, Lachenbruch PA, et al: Relationship of parental smoking and gas cooking to respiratory disease in children. *Chest* 1983; 84:662-668.
 49. Vedal S, Schenker MB, Samet JM, et al: Risk factors for childhood respiratory disease: analysis of pulmonary function. *Am Rev Respir Dis* 1984; 130:187-192.
 50. Melia RJ, Florey CD, Altman DG: Association between gas cooking and respiratory disease in children. *Br Med J* 1977; 2:149-152.
 51. Melia RJW, Florey CduV, Chinn S: The relation between respiratory illness in primary school children and the use of gas for cooking: I. Results from a national survey. *Int J Epidemiol* 1979; 8:333-338.
 52. Melia RJW, Florey CduV, Morris RW, et al: Childhood respiratory illness and the home environment: I. Relations between nitrogen dioxide, temperature and relative humidity. *Int J Epidemiol* 1982; 11:155-163.
 53. Melia RJW, Florey CduV, Morris RW, et al: Childhood respiratory illness and the home environment: II. Association between respiratory illness and nitrogen dioxide, temperature and relative humidity. *Int J Epidemiol* 1982; 11:164-169.
 54. Ogston SA, Florey CduV, Walker CHM: The Tayside infant morbidity and mortality study: Effect on health of using gas for cooking. *Br Med J* 1985; 290:957-960.
 55. Schenker MB, Samet JM, Speizer FE: Risk factors for childhood respiratory disease: The effect of host factors and home environmental exposures. *Am Rev Respir Dis* 1983; 128:1038-1043.
 56. Dockery DW, Ware JH, Ferris BG Jr, et al: Change in pulmonary function in children associated with air pollution episodes. *J Air Pollut Control Assoc* 1982; 32:937-942.
 57. Dassen W, Brunekreef B, Hoek G, et al: Decline in children's pulmonary function during an air pollution episode. *J Air Pollut Control Assoc* 1986; 11:1223-1227.
 58. Ware JH, Ferris BG Jr, Dockery DW, et al: Effects of ambient sulfur oxides and suspended particles on respiratory health of preadolescent children. *Am Rev Respir Dis* 1986; 133:834-842.
 59. Chapman RS, Calafiore DC, Hasselblad V: Prevalence of persistent cough and phlegm in young adults in relation to long-term ambient sulfur oxide exposure. *Am Rev Respir Dis* 1985; 132:261-267.
 60. Dodge R, Solomon P, Moyers J, et al: A longitudinal study of children exposed to sulfur oxides. *Am J Epidemiol* 1985; 121:720-736.
 61. Honicky RE, Osborne JS III, Akpom CA: Symptoms of respiratory illness in young children and the use of wood-burning stoves for indoor heating. *Pediatrics* 1985; 75:587-593.
 62. National Research Council: The physicochemical nature of sidestream smoke and environmental tobacco smoke, in *Environmental Tobacco Smoke—Measuring Exposures and Assessing Health Effects*. Washington, DC, National Academy Press, 1986, pp 25-53.
 63. Vainio H, Hemminki K, Wilbourn J: Data on the carcinogenicity of chemicals in the IARC Monographs programme. *Carcinogenesis* 1985; 6:1653-1665.

64. Spengler JD, Dockery DW, Turner WA, et al: Long-term measurements of respirable sulfates and particles inside and outside homes. *Atmos Environ* 1981; 15:23-30.
65. Harlap S: A time-series analysis of the incidence of Down's syndrome in West Jerusalem. *Am J Epidemiol* 1974; 99:210-217.
66. Leeder SR, Corkhill R, Irwig LM, et al: Influence of family factors on the incidence of lower respiratory illness during the first year of life. *Br J Prev Soc Med* 1976; 30:203-212.
67. Rantakallio P: Relationship of maternal smoking to morbidity and mortality of the child up to the age of five. *Acta Paediatr Scand* 1978; 67:621-631.
68. Fergusson DM, Hons BA, Horwood LJ: Parental smoking and respiratory illness during early childhood: A six-year longitudinal study. *Pediatr Pulmonol* 1985; 1:99-106.
69. Pullan CR, Hey EN: Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytal virus in infancy. *Br Med J* 1982; 284:1667-1669.
70. Chen Y, Li W, Yu S: Influence of passive smoking on admissions for respiratory illness in early childhood. *Br Med J* 1986; 293:303-306.
71. Colley JRT: Respiratory symptoms in children and parental smoking and phlegm production. *Br Med J* 1974; 2:201-204.
72. Bland M, Bewley BR, Pollard V, et al: Effect of children's and parents' smoking on respiratory symptoms. *Arch Dis Child* 1978; 53:100-105.
73. Weiss ST, Tager IB, Speizer FE, et al: Persistent wheeze: Its relation to respiratory illness, cigarette smoking, and level of pulmonary function in a population sample of children. *Am Rev Respir Dis* 1980; 122:697-707.
74. Charlton A: Children's coughs related to parental smoking. *Br Med J* 1984; 288:1647-1649.
75. Burchfiel CM III: *Passive Smoking, Respiratory Symptoms, Lung Function, and Initiation of Smoking in Tecumseh, Michigan*, PhD dissertation. University of Michigan, Detroit, 1984.
76. Berkey CS, Ware JH, Dockery DW, et al: Indoor air pollution and pulmonary function growth in preadolescent children. *Am J Epidemiol* 1986; 123:250-260.
77. Dahms TE, Bolin JF, Slavin RG: Passive smoking: Effects on bronchial asthma. *Chest* 1981; 80:530-534.
78. Shephard RJ, Collins R, Silverman F: "Passive" exposure of asthmatic subjects to cigarette smoke. *Environ Res* 1979; 20:392-402.
79. Wiedemann HP, Mahler DA, Loke J, et al: Acute effects of passive smoking on lung function and airway reactivity in asthma subjects. *Chest* 1986; 89:180-185.
80. National Research Council: Effects of exposure to environmental tobacco smoke on lung function and respiratory symptoms, in *Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects*. Washington, DC, National Academy Press, 1986, pp 182-222.
81. US Environmental Protection Agency: *Preliminary Indoor Air Pollution Information Assessment, Appendix A*. Washington, DC, EPA report no. EPA 600/8-87/014, 1987.
82. Committee on Environmental Hazards of the American Academy of Pediatrics. Asbestos exposure in schools. *Pediatrics* 1987; 79:301-305.
83. Germine M: Asbestos in play sand. *N Engl J Med* 1986; 315:891.
84. Doll R, Peto J: Effects on health of exposure to asbestos, in *Health and*

- Safety Commission. London, Her Majesty's Stationery Office, 1985, pp 31–48.
85. Brenner J: Malignant mesothelioma in children: Report of seven cases and review of the literature. *Med Pediatr Oncol* 1981; 9:367–373.
 86. Huncharek M: The biomedical and epidemiological characteristics of asbestos-related diseases: A review. *Yale J Biol Med* 1986; 59:435–451.
 87. Sterling DA: Volatile organic compounds in indoor air: An overview of sources, concentrations, and health effects, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985, pp 387–402.
 88. Wallace LA: Personal exposures, indoor and outdoor air concentrations, and exhaled breath concentrations of selected volatile organic compounds measured for 600 residents of New Jersey, North Dakota, North Carolina, and California. *Toxicol Environ Chem* 1986; 12:215–236.
 89. Wallace LA, Pellizzari ED, Hartwell TD, et al: Total exposure assessment methodology (TEAM) study: Personal exposures, indoor-outdoor relationships, and breath levels of volatile organic compounds in New Jersey, in Berglund B, Berglund U, Lindvall T, et al (eds): *Indoor Air Quality: Papers from the Third International Conference on Indoor Air Quality and Climate, August 1984, Stockholm, Sweden*. *Environ Int* 1986; 12:369–387.
 90. Savage EP, Keefe TJ, Wheeler HW, et al: Household pesticide usage in the United States. *Arch Environ Health* 1981; 36:304–309.
 91. Jensen J: *Potential Pesticide Indoor Air Pollutants*, memo. US Environmental Protection Agency, November 10, 1980.
 92. Lewis R, MacLeod K: Portable sampler for pesticides and semivolatile industrial organic chemicals in air. *Anal Chem* 1982; 54:310–315.
 93. Stevens FD: Sampling methodology for airborne semivolatile organic pollutants using polyurethane foam, PhD dissertation. University of Texas School of Public Health, Houston, June 1984.
 94. National Research Council: *Formaldehyde and Other Aldehydes*. Washington, DC, National Academy Press, 1981.
 95. Molhave L: Volatile organic compounds as indoor air pollutants, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985, pp 403–414.
 96. 3M Organic Vapor Monitor Part #1876-4. Lab Safety Supply, PO Box 1368, Janesville, WI 53547-1368.
 97. Nelms LH, Mason MA, Tichenor BA: The effects of ventilation rates and product loading on organic emission rates from particleboard, in *Proceedings of the ASHRAE Conference: Managing Indoor Air for Health and Energy Conservation*, IAQ 1986, April, Atlanta, American Society of Heating, Refrigerating, and Air-Conditioning Engineers Inc, 1986, pp 469–485.
 98. Upton AC: Biological basis for assessing carcinogenic risks of low-level radiation. *Carcinogenesis* 1985; 10:381–401.
 99. Nero AV Jr: Indoor concentrations of radon-222 and its daughters: Sources, range and environmental influences, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985, pp 43–67.
 100. Consumer Federation of America: *Indoor Air News*, summer 1987; 3(3).
 101. Robins J: Risk assessment: Exposure to environmental tobacco smoke and lung cancer, in National Research Council: *Environmental Tobacco Smoke*:

- Measuring Exposures and Assessing Health Effects*. Washington, DC, National Academy Press, 1986, pp 294–337.
102. Steinhausler F: European radon surveys and risk assessment, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985, pp 109–129.
 103. Wilson R, Crouch EAC: Risk assessment and comparisons: An introduction. *Science* 1987; 236:267–270.
 104. Transmission/distribution health and safety, in *Transmission/Distribution Health and Safety Report*. Minneapolis, Interdisciplinary Environmental Associates, Inc, 1986, vol 3, p 1.
 105. Goodman R, Henderson AS: Sine waves enhance cellular transcription. *Bioelectromagnetics* 1986; 7:23–29.
 106. Phillips JL, Winters WD, Rutledge L: In vitro exposure to electromagnetic fields: Changes in tumour cell properties. *Int J Radiat Biol* 1986; 49:463–469.
 107. Luben RA, Cain CD, Chen MCY, et al: Effects of electromagnetic stimuli on bone and bone cells in vitro: Inhibition of responses to parathyroid hormone by low-energy low-frequency fields. *Proc Natl Acad Sci USA* 1982; 79:4180–4184.
 108. Blackman CF, Benane SG, Joines WT: Effects of ELF (1–120 Hz) and modulated (50 Hz) RF fields on the efflux of calcium ions from brain tissue in vitro. *Bioelectromagnetics* 1985; 6:1–11.
 109. Joines WT, Blackman CF, Spiegel RJ: Specific absorption rate in electrically coupled biological samples between metal plates. *Bioelectromagnetics* 1986; 7:163–1976.
 110. Thomas JR, Schrot J, Liboff AR: Low-intensity magnetic fields alter operant behavior in rats. *Bioelectromagnetics* 1986; 7:349–357.
 111. Wertheimer N, Leeper E: Electrical wiring configuration and childhood cancer. *Am J Epidemiol* 1979; 109:273–284.
 112. Tomenius L: 50-Hz electromagnetic environment and the incidence of childhood tumors in Stockholm County. *Bioelectromagnetics* 1986; 7:191–207.
 113. Wertheimer N, Leeper E: Possible effects of electric blankets and heated waterbeds on fetal development. *Bioelectromagnetics* 1986; 7:13–22.
 114. Houk VN, Kent DC, Baker JH, et al: The epidemiology of tuberculosis infection in a closed environment. *Arch Environ Health* 1968; 16:26–35.
 115. LaForce FM: Airborne infections and modern building technology, in *Proceedings of the 3rd International Conference on Indoor Air Quality and Climate*. Stockholm, Sweden, Council for Building Research, 1984, vol 1, pp 109–127.
 116. Sarubbi FA, Kopf HB, Wilson MB, et al: Increased recovery of *Aspergillus flavus* from respiratory specimens during hospital construction. *Am Rev Respir Dis* 1982; 125:33–38.
 117. Kyriakides GK, Zinneman HH, Hall WH, et al: Immunologic monitoring and aspergillosis in renal transplant patients. *Am J Surg* 1976; 131:246–252.
 118. Fraser DW, Tsai TR, Orenstein W, et al: Legionnaire's disease: Description of an epidemic of pneumonia. *N Engl J Med* 1977; 297:1189–1203.
 119. Glick TH, Gregg MB, Berman B, et al: Pontiac fever: An epidemic of unknown etiology in a health department: I. Clinical and epidemiologic aspects. *Am J Epidemiol* 1978; 107:149–160.
 120. Dondero TJ, Rendtorff RC, Mallison GF, et al: An outbreak of Legionnaires'

- disease associated with an air-conditioning cooling tower. *N Engl J Med* 1980; 203:365–370.
121. Cordes LG, Wiesenthal AM, Gorman GW, et al: Isolation of *Legionella pneumophila* from hospital shower heads. *Ann Intern Med* 1981; 94:195–197.
 122. Meyer RD: *Legionella* infections: A review of five years of research. *Rev Infect Dis* 1983; 5:258–278.
 123. Tobin JO, Beare J, Dunnill MS, et al: Legionnaires' disease in a transplant unit: Isolation of the causative agent from shower baths. *Lancet* 1980; 2:118–121.
 124. Salvaggio JE: Diagnosis and management of hypersensitivity pneumonitis. *Hosp Pract* 1980; 93–103.
 125. Johnson CL, Bernstein IL, Gallagher JS, et al: Familial hypersensitivity pneumonitis induced by *Bacillus subtilis*. *Am Rev Respir Dis* 1980; 122:339–348.
 126. Edwards JH, Cockcroft A: Inhalation challenge in humidifier fever. *Clin Allergy* 1981; 11:227–235.
 127. Yamazaki M, Suzuki S: Toxicology of tremorgenic mycotoxins, fumitremorgin A and B, in Chambers PL, Gehring P, Sakai F (eds): *New Concepts and Developments in Toxicology*. New York, Elsevier Science Publishers, 1986, p 273.
 128. Jarvis BB: Potential indoor air pollution problems associated with macrocyclic trichothecene producing fungi, in *Significance of Fungi in Indoor Air: Report of a Working Group*. Ontario, Canada, Health and Welfare Canada Working Group on Fungi and Indoor Air, 1986.
 129. Burge HA: Indoor sources for airborne microbes, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985, pp 139–148.
 130. Offerman F, Sextro R, Fisk W, et al: Control of respirable particles and radon progeny with portable air cleaners, report LBL-16659. Berkeley, Calif, Lawrence Berkeley Laboratory, February 1984.
 131. Mansmann HC Jr: Environmental control, in Middleton E Jr, Reed CE, Ellis EF (eds): *Allergy Principles and Practices*. St Louis, CV Mosby Co, 1978, vol 2, pp 957–964.
 132. Patterson R, Beltrani VS, Singal M, et al: Creating an indoor environmental problem from a nonproblem: A need for cautious evaluation of antibodies against hapten-protein complexes. *NER Allergy Proc* 1985; 6:135–140.
 133. Mage DT, Gammage RB: Evaluation of changes in indoor air quality occurring over the past several decades, in Gammage RB, Kaye SV, Jacobs VA (eds): *Indoor Air and Human Health*. Chelsea, Mich, Lewis Publishers, Inc, 1985, pp 5–36.
 134. Cote WA, Wade WA III, Yocum JE: *A Study of Indoor Air Quality*. Washington, DC, US Environmental Protection Agency, EPA Report no. EPA 650/4-74-042, September 1974.
 135. Yocum JE, Clink WL, Cote WA: Indoor/outdoor air quality relationships. *J Air Pollut Control Assoc* 1971; 21:251–259.
 136. Burchfiel CM, Higgins MW, Keller JB, et al: Passive smoking in childhood: Respiratory conditions and pulmonary function in Tecumseh, Michigan. *Am Rev Respir Dis* 1986; 133:966–973.

Aluminum: A Pediatric Overview

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The clinical syndrome of aluminum toxicity was first recognized in 1972 as the dialysis encephalopathy syndrome (DES) in patients undergoing hemodialysis.¹ In 1976, brain tissue of adult patients dying from a progressive neurologic disorder while on hemodialysis was reported to contain large quantities of Al.² In 1977, we first reported five children who in the absence of dialysis developed a progressive encephalopathy similar to that observed in adults.^{3, 4} Each child had a congenital form of renal disease, developed renal failure within a few months of birth, and received large amounts of aluminum-containing phosphate binders (ACPBs) at a dose of 240 to 800 mg/kg/dl for a period of months to years. Eventually, plasma Al levels were found to be increased in two of these children.⁵

Although the initial reports involving adult patients focused on alterations in CNS function, subsequent studies delineated additional clinical problems related to Al. Problems related to Al occur most often in patients with renal insufficiency and include encephalopathy, aluminum bone disease (ABD), and anemia. Al may also play a role in some degenerative CNS diseases and in osteopenia associated with parenteral alimentation. Articles dealing with Al now exist in the adult and mature animal literature, but fewer studies involving children and growing animals are available; however, the role of Al in causing childhood disease is becoming clearer.⁶ This article provides an overview of what is known about Al exposure, and its pathophysiology as a heavy metal and reviews data relating Al to specific diseases from a pediatric perspective.

Aluminum: A Heavy Metal

Distribution

Al is the third most abundant element and most plentiful metal in the outer crust of the planet earth, occurring as various aluminosilicates. Free Al concentrations in natural water are low because Al hydroxide forms stable

structures. Al concentration in natural fresh water is in the range of 10^{-5} to 10^{-6} molar except when the pH of water is less than 4.5 or greater than 9.0, when its concentration increases. Al forms strong complexes with oxalates and fluorides and it polymerizes as pH changes; complex Al compounds attach to negatively charged organic solutes. Alum (Al sulfate) has been used for many years as a flocculent to clarify turbid water. In most aqueous solutions, Al exists in ionized and bound forms.⁷

Measurement

The development of atomic absorption (AA) photometry made possible routine measurements of Al at concentrations found in biologic specimens.⁸ Reproducible, accurate measurements, however, have been and remain difficult to achieve. Standard AA flame spectrophotometry is adequate for measuring urinary Al levels, while flameless AA photometry, which utilizes a graphite furnace, is needed to measure blood levels of Al. Technical difficulties involved in measuring Al cause many problems in studying Al in disease and explain the wide range of values observed in diseased patients as well as normal individuals.

Methodology

Sample contamination is a potentially serious problem. Plastic syringes, stainless steel needles, and plastic blood containers (B-D red top containers no. 6430) must be used to collect samples, because glass containers carry significant amounts of Al.⁹ Some heparin preparations contain Al. Samples may be stored in Falcon plastic tubes and transported at room temperature. Pipettes and containers used to prepare reagents need to be washed with nitric acid or ethylenediamine tetraacidic acid, and Al-free reagents must be used. An environmental laminar flow hood reduces contamination from dust, cigarette smoke, and other environmental agents. Water used for dilutions, etc., should have a resistivity of greater than 18 megohms.

Microliter samples are injected into a graphite furnace in the path of an optical beam of an Al hollow cathode lamp. The furnace performs three sequential functions. First, the sample is dried and solvent removed at temperatures ranging from 80 to 300 C. Second, the sample is ashed at 700 to 1,400 C to remove inorganic and organic matrices. Third, the sample is atomized at 2,700 to 2,800 C to generate free atoms. The height of the absorption peak is related to the amount of Al.¹⁰ The major problem in measuring blood samples is the influence of the matrix formed when samples are rapidly heated to temperatures greater than 2,500 C.¹¹ Both organic (proteins and glucose) and inorganic (mono and divalent ions) compounds can enhance or depress the Al signal. Diluting or concentrating serum does not routinely result in proportional changes in Al measurements because of matrix effects. Chemical and physical problems also interfere with Al determinations. Chemical interference may be due either to the formation of compounds that do not decompose or to the formation of volatile compounds that contain Al. Physical problems include background absorption, surface tension, and viscosity. These problems are overcome by careful attention to temperature settings, appropriate

dilution of samples, drying times appropriate for the volume of sample injected, and the use of matrix modifiers such as nitric acid, Triton X-100, etc. The preparation of the graphite tube is equally important. Pyrolytically coated tubes, tubes soaked in molybdenum, and tubes pretreated with tantalum pentoxide have been successfully used.

Based on the quantity of Al required to change absorbance of Al by 1%, reported Al sensitivities range from 13 to 40 pg with a coefficient of variation of 2.9% to 7.0%.¹² Accuracy is usually determined by an inhouse sample to which a known quantity of Al has been added. Precision is ascertained by measuring the variation between repeated measurements and between batches. It is suggested that all samples from a given patient be processed by the same laboratory and that consideration be given to correcting for interassay differences.

Aluminum Physiology

Presently, the only available tools to study Al physiology are balance studies and tissue measurements. The average intake of Al in normal adults ranges from 3 to 5 mg/day. In normal adults, 98% of ingested Al is not absorbed.¹³ Most absorbed Al is excreted by the kidney if the glomerular filtration rate (GFR) is normal.¹⁴ Renal clearances of Al range from 2% to 50% of normal GFR. Urinary concentrations are indicators of Al intake. Usable radiolabeled isotopes of Al are not available to study Al physiology in humans.¹⁵ In normal adults, urinary Al excretion increases from 15 mg/day to 250 to 350 mg/day after ACPBs are ingested.¹⁶ The low renal clearance of Al at low serum Al levels may be related to binding of Al by a saturable plasma component at Al blood levels less than 200 $\mu\text{g/L}$.¹⁷ Al is tightly tissue bound and exists in blood in bound and free forms. Al binds mainly to serum transferrin and albumin¹⁸; the biologic significance of this binding is unknown. Both transferrin and albumin may play a role as Al detoxicants; theoretically, lowered serum levels of either may increase the risk of Al toxicity.

Balance studies in normal adults indicate that 0.02% to 0.10% of ingested Al is retained; the remaining Al, which is absorbed, is excreted in the urine. Al is absorbed in its ionized form, which is affected by gastric pH. The solubility of $\text{Al}(\text{OH})_3$ increases 100,000-fold when the pH falls from 6.5 to 5.5.¹⁹ Additional factors leading to an increased gut absorption of Al include uremic-induced changes in parathyroid hormone (PTH) and vitamin D metabolism and perhaps low calcium states.¹⁵ A common pathway for gut absorption of Al, lead, iron, cadmium, and cobalt may exist.¹⁵

Experimental studies have expanded our understanding of how Al is handled once it enters the body. Following an initial rapid intravenous infusion of 1 mg/kg elemental Al to unanesthetized dogs, plasma half-life of Al was 276 minutes with a distribution volume of approximately 6.0% of body weight.²⁰ The quantity of Al excreted by the kidney was 10% to 21% after 150 minutes. Mean serum Ca increased from 9.4 to 11.1 mg/dl and immunoreactive PTH (iPTH) fell by 27%. Plasma clearance exceeded renal clearance, suggesting tissue deposition. Other studies report that Al

given parenterally is not excreted in bile or via the intestines.²¹ Chronic Al infusions given over 3 to 5 weeks were associated with the development of hypercalcemia and depressed renal function, which by itself was not felt capable of explaining the changes in serum Ca. Also, since iPTH remained normal or increased, it was believed that serum ionized Ca levels had not changed. Because serum $1,25(\text{OH})_2\text{D}$ fell while $25(\text{OH})_2\text{D}$ did not change, a direct effect of Al on $1,25(\text{OH})_2\text{D}$ synthesis by proximal tubules was suggested.²²

In rats, a 2-hour infusion of Al, 0.4 mg Al per 100 gm of body weight, increased mean total Ca from 9.7 to 12.7 mg/dl while ionized and ultrafilterable Ca decreased from 5.1 to 4.05 mg/dl and from 5.4 to 4.53 mg/dl, respectively.²³ The proposed mechanism for these changes is that Al increased bound Ca either by directly complexing Ca or by complexing with already bound Ca. Consequently, concentrations of ionized Ca fell and additional ionized Ca was mobilized from Ca stores to replenish the lost ionized Ca.

Al also elevates serum Ca in vitamin-D-deficient chicks, further supporting the hypothesis that Al-related hypercalcemia results from an increased release of Ca from bone.²⁴

General Mechanisms of Aluminum Toxicity

Metals must bind to cofactors or proteins to become biologically active.²⁵ It appears that Al interferes with reactions that involve cations (Ca, Mg, Fe) which have similar properties. Although the biochemical basis of Al intoxication remains incompletely understood, Al is known to interact with a number of proteins and cofactors involved in intermediary metabolism. Al combines with adenosine triphosphate (ATP) to form Al-ATP, a competitive inhibitor of hexokinase.²⁶ Other enzymes inhibited by Al include catechol-O-methyl transferase, ceruloplasmin (ferroxidase), cholinesterase, choline acetyltransferase, glycerokinase, and Mg-adenosine triphosphatase. Al also inhibits calmodulin^{27, 28} and erythrocyte dihydropteridine reductase and activates adenylate cyclase and d-aminolevulinic dehydratase.

Aluminum Exposure

Currently, the major sources of Al exposure for patients with renal failure are ACPBs (Tables 1 and 2), Al-containing additives (Table 3) used in preparing intravenous solutions, possibly Al-containing salts used in infant formula preparation (see Table 3), and Al absorbed from water used in dialysis.²⁹ Foods and cookware may also minimally contribute to the total Al burden. It is difficult to determine the precise Al burden that patients with Al-related diseases receive. For example, patients often do not ingest all of their prescribed ACPBs. Less well appreciated is that many antacids are combination preparations and many such agents contain $\text{Al}(\text{OH})_3$ in amounts ranging from 6 to 138 mg per tablet or per teaspoon. The Al

TABLE 1.
Aluminum Content of Various Drugs

Product	How Supplied	Elemental Al in mg
Alu-Cap (Riker)	Capsules: 475 mg	164 mg
Dialume (Armour)	Capsules: 500 mg	173 mg
Alagel (Century)	Suspension: 320 mg/5 ml	111 mg
Amphojel (Wyeth)	Tablets: 300 mg, 600 mg	104 mg
	Suspension: 320 mg/5 ml	207 mg
		111 mg
Aluminum hydroxide (Schein)	Tablets: 500 mg	173 mg
Alu-Tab (Riker)	Tablets: 600 mg	207 mg
Aluminum hydroxide gel, USP (Various)	Suspension: 200 mg Al ₂ O ₃ /5 ml	106 mg Al
Concentrated aluminum hydroxide (Roxane)	Suspension: 600 mg	207 mg/5 ml
Alternagel (Stuart)	Liquid: 600 mg/5 ml	207 mg/5 ml
Phosphaljel	Suspension: 233 mg/5 ml	51 mg/5 ml
Rolaid Antacid	Tablets, chewable: 334 mg	63 mg
Basaljel	Capsules: Equivalent to 500 mg aluminum hydroxide	173 mg
	Swallow tablets: Equivalent to 500 mg aluminum hydroxide	173 mg
	Suspension: Equivalent to 400 mg aluminum hydroxide per 5 ml	138 mg/5 ml
	Extra strength suspension: Equivalent to 1,000 mg aluminum hydroxide/5 ml	346 mg/5 ml
Lowsium	Tablets: 480 mg	66 mg Al
	Suspension: 480 mg	66 mg Al/5 ml
Riopan	Suspension: 540 mg/5 ml; (140 mg Al ₂ O ₃)	74 mg Al/5 ml
Sucrafate	Tablets: 100 mg	182–207 mg/Al per tab

content of a few of the better known combination antacids follow: Maalox, Mylanta, and Gelusil, number 1 and 2 tablets contain 69 and 138 mg of Al; Nephrox contains 111 mg of Al; and Di-Gel liquid contains 98 mg per 5 ml.

Plasma Al concentrations rise twofold to threefold after 3 days of in-

TABLE 2.
Percent Elemental Aluminum Contained in
Aluminum-Containing Drugs

Aluminum Salt	% Al Contained in Salt
Aluminum hydroxide	34.58
Aluminum phosphate	22.12
Dihydroxy Al carbonate	18.74
Bismuth aluminate	20.97
Aluminum oxide	52.91
	% yield of Al oxide
Aluminum glycinate	35.5–38.5
Aluminum carbonate	5.1
Hydroxymagnesium aluminate (magaldrate)	18–26

gesting 2.2 gm of ACPBs, $\text{Al}(\text{OH})_3$, AlCO_3 , or dihydroxyaluminum aminoacetate.¹⁶ Urinary Al excretion also increases considerably but not to values that can account for all the ingested Al being absorbed and excreted.

The nature of the anion bound to Al may influence tissue deposition. Al citrate may increase tissue Al accumulation.³⁰ Some patients have been reported to develop acute fatal encephalopathy after receiving citrate in addition to $\text{Al}(\text{OH})_3$. However, it has been reported that Al accumulation in the femur and brain of weanling rats did not differ when the source of Al was $\text{Al}(\text{OH})_3$ or Al citrate.³¹

The Al content of many agents has only recently been systematically evaluated. For example, the ingestion of sucralfate (which contains 21% Al by weight and is used to treat dyspepsia) raises plasma Al and can lead to Al toxicity in patients with end-stage renal disease (ESRD).³²

A number of agents used to prepare parenteral nutritional solutions and systemically administered agents are now known to contain significant amounts of Al. Also, the ingestion of Al-containing infant formulas may lead to Al toxicity in infants with renal insufficiency.³³ The source of Al is from Al salts used in preparing salts added to the formula. Even though breast milk normally contains little Al, the possibility exists that nursing mothers exposed to Al-containing agents may develop elevated breast milk Al. Human data are not currently available. Dairy cows and lactating rabbits have slightly increased milk Al levels after being exposed to Al. The increase is rather small, and no cumulative effect is found after a month in newly born rabbits despite doses of Al to their does that lead to an in-

TABLE 3.
Aluminum Content of IV Solutions, Additives,
Breast Milk, and Infant Formulas*

Additives and Solutions (Manufacturer)	Aluminum/Content ($\mu\text{g/L}$)
5% Albumin (Armour)	1,108 \pm 348
5% Albumin (Cutter)	332 \pm 92
25% Albumin (Cutter)	2,570†
5% Albumin (Hyland/Travenol)	163 \pm 96
25% Albumin (Hyland/Travenol)	448†
5% Albumin (Red Cross)	391 \pm 51
25% Albumin, serum albumin	1,822 \pm 2,503
TPW 10% casein hydrolysate	5,056 \pm 335
10% calcium gluconate	
5% dextrose	72 \pm 1
Heparin, 1,000 units/mL	684 \pm 761
Potassium chloride, 3,000 mmol/L	6†
Potassium phosphate, 3,000 mmol/L	16,598 \pm 1,801
Sodium chloride, 4,000 mmol/L	6 \pm 4

Infant Formula	Aluminum Content ($\mu\text{g/L}$)
Cow's milk-based formula	
20 kcal/30 ml	266 \pm 92
"Premature" (24 kcal/30 ml)	699 \pm 321
Enfamil formulas (20 kcal and 24 kcal/30 ml; "Neonatal")	124 – 391
Glucose water	20†
Human milk	9.9 \pm 6.87
Similac PM 60/40 (20 kcal/30 ml)	232 \pm 60
Similac formulas (20 kcal and 24 kcal/30 ml; Special Care)	126 – 294
Soy-based formula	1,478 \pm 103

*Adapted from Freundlich M, Zilleruelo G, Abitbol C, et al: Infant formula as a cause of aluminum toxicity in neonatal uraemia. *Lancet* 1985; 2:527–529. Ott SM, Maloney NA, Klein GL, et al: Aluminum is associated with low bone formation in patients on chronic parenteral nutrition. *Ann Intern Med* 1983; 98:910–914. Sedman AB, Klein GL, Merritt RJ, et al: Evidence of aluminum loading in infants receiving intravenous therapy. *N Engl J Med* 1985; 312:1337–1343. Milliner DS, Shinaberger JH, Shubman P, et al: Inadvertent aluminum administration during plasma exchange due to aluminum contamination of albumin replacement solutions. *N Engl J Med* 1985; 312:165.

†Single determinations.

creased tissue Al content.³⁴ Despite the unlikelihood of infants developing Al intoxication from human breast milk, it seems prudent to advise nursing mothers to avoid the regular usage of Al-containing medicinals. It has been suggested that the intestinal tract of weanling rats is more susceptible to absorbing orally administered Al.³⁵

Some foods contain Al, for example, rhubarb, oatmeal, and carrots, and have an aluminum content that varies from 0.43 to 0.55 mg per 100 gm of food.³⁶ Cooking of acidic or basic, compared with neutral, foods in cast aluminum cookware is corrosive and increases the available Al.³⁷ The residual Al in food cooked in Al cookware can increase twofold to threefold (i.e., from 0.89 to 1.62 mg per 100 gm food). It is also possible to leech Al from Al foil during the cooking process.³⁸ Thus, water in which food is cooked may have an increased Al content. Ingestion of this water can increase the Al burden. Data on Al leeching from cookware after prolonged use are not available, but the prolonged use of Al cookware may contribute to a long-term accumulation of Al. Data on the Al content, if any, of soft drinks in Al cans are not available. The amount of Al obtained from either foods or cookware is of minimal clinical significance when compared with other sources of Al in patients with renal insufficiency.

Marked variations in Al concentrations can occur over time in the same water supply. Changes in industrial dumping are unpredictable as is the washoff into water supplies of natural sources of Al and the addition of alum (Al sulfate) as a flocculent to clear municipal water supplies. The Al content of water used to prepare dialysate varied considerably prior to the widespread adaptation of water-purifying systems.

Interpretation of Blood Aluminum Levels

Differences between whole blood and plasma Al are insignificant. Reported normal values range from 2.5 to 1,800 $\mu\text{g/L}$,¹¹ most likely a reflection of different methodologies and contamination of samples at various steps. Current data suggest that normal children and adults should have blood Al concentrations less than 10 to 15 $\mu\text{g/L}$. (Division of μg or mg by 27 converts Al values to μmole or mmole .) The inherent difficulty in comparing values obtained from different laboratories is illustrated by the results reported from three commercial laboratories that received aliquots from single samples. Obtained prior to and after administration of desferrioxamine (DFO), samples differed by a factor of 9- to 10-fold and 1.5-fold, respectively.³⁹ The relative increases above baseline values were not similar after giving DFO.

The timing of drawing of blood samples in patients receiving ACPBs influences values for blood Al. In a group of 67 patients, mean plasma Al levels fell from 132 to 99 $\mu\text{g/L}$ in 21 who had discontinued ACPBs for 24 hours. In 18 patients given DFO, a positive response occurred in only three.⁴⁰ Such data suggest that the need for additional diagnostic testing may be avoided if screening Al samples are collected after ACPBs are

discontinued. Furthermore, serum Al levels are known to change after oral administration of ACPBs in patients undergoing continual ambulatory peritoneal dialysis (CAPD). In a study of 10 adults on CAPD, serum Al levels measured hourly for 8 hours after the oral ingestion of 1,800 mg of aluminium chlorohydrate increased progressively for 5 hours and returned to baseline by 24 hours.⁴¹

Blood values are more helpful in determining the risk of acute rather than chronic toxicity. Blood levels and intracellular effects (plasma levels and bone Al) correlate poorly in both adults⁴² and children.⁴³ However, the degree of risk from Al does seem to be grossly related to plasma Al levels, particularly in steady state situations. For example, ongoing exposure to ACPBs for prolonged periods increases the risk of Al toxicity since blood Al levels tend to rise over time. The duration and nature of exposure are equally important.

Relationship Between Blood Aluminum Levels and Aluminum Toxicity in Children and Adults

A few examples of the risks associated with Al exposure are considered here. Other sections contain additional data relating blood Al levels to specific tissue accumulation of Al.

In children with chronic renal failure, serum Al levels are positively related to the dose of elemental Al ingested (Fig 1).^{44, 45} Available data suggest that serum Al levels greater than 100 $\mu\text{g/L}$ are clearly associated with an increased risk of developing Al toxicity in children,⁴⁴ and many examples of Al toxicity have been documented at lower serum levels. One study compared six children with Al levels greater than 100 mg/L to 25 children with lower levels.⁴⁵ Five of the six had Al staining on bone biopsy, one developed encephalopathy, and four of five and five of five had motor and social delay. In the group of 25 children with serum Al levels less than 100 $\mu\text{g/L}$, 0/7 had Al on bone biopsy, 0/25 had encephalopathy, and 1/3 had motor delay.

Many patients undergoing dialysis develop hyperaluminemia from ACPBs and Al in the dialysate. Serum Al values in patients on peritoneal dialysis vary considerably (Table 4). In a recent report assessing risk factors for hyperaluminemia in 51 adults on CAPD, serum Al correlated positively with ACPB intake in the previous 6 months, total intake of elemental Al, duration of CAPD, and serum phosphate levels.⁴⁶ Serum Al levels in adults undergoing peritoneal dialysis are lower in those not given ACPBs.⁴⁷

Serum Al levels in children ingesting ACPBs while undergoing dialysis are almost always elevated. Serum Al levels were measured twice (mean duration of CAPD, 7.9 and 16.6 months) in 16 children aged 1.5 to 14.2 years undergoing CAPD (Table 5).⁴⁸ Final serum Al levels (which were similar to initial levels) correlated with the child's mean oral intake of Al ($r = 0.87$; $P < 0.001$). Most important, inverse correlations existed between

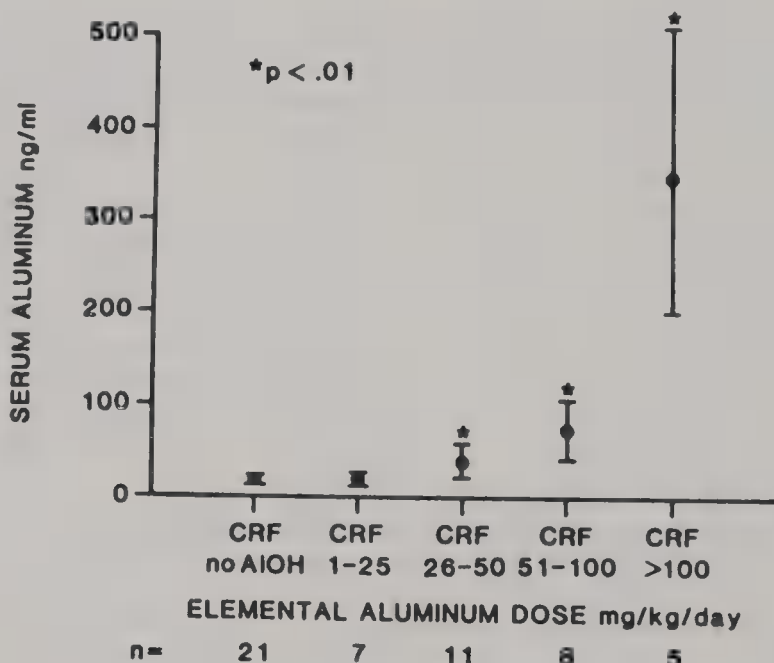


FIG 1.

The relationship between dose of elemental Al and serum Al levels in children with chronic renal failure. Significantly elevated serum levels ($P < 0.01$) were found in children ingesting more than 25 mg/kg/day. (From Sedman AB, Miller NL, Warady BA, et al: Aluminum loading in children with chronic renal failure, *Kidney Int* 1984; 26:201-204. Used by permission.)

weight ($r = -0.68$; $P < 0.01$) and age ($r = -0.67$; $P < 0.01$) and serum Al levels. The younger children were given higher doses of ACPBs.

The dialysis modality utilized may impact upon serum Al levels; varied results have been reported. In 37 children aged 14 months to 19 years undergoing CAPD or hemodialysis, serum Al levels were lower in those on CAPD^{5, 49} (Table 6), perhaps due to a decreased phosphate intake and/or a reduced need for ACPBs. Continuous (CAPD) as compared with intermittent forms of dialysis removes more phosphate. Serum Al levels did not correlate with midrange iPTH levels. In 17 children aged 0.5 to 18.0 years, plasma Al levels were lowest in those on hemodialysis, progressively higher in those on peritoneal dialysis, and highest in those not yet on dialysis;⁴³ serum levels were related to the intake of ACPBs ($r = 0.60$; $P < 0.01$).

In 185 adult patients treated with ACPBs and dialyzed for 4 to 95 months with a reverse osmosis water-purifying system (presumed dialysate Al level, < 10 – $15 \mu\text{g/L}$), correlation was observed between unstimulated (no DFO) plasma Al levels and the duration of dialysis.⁵⁰ Plasma Al levels after DFO increased more the longer one had been on dialysis. These data indicate that the body burden of Al had progressively increased. No correlation could be found between stimulated Al levels and clinical symptoms of Al toxicity, measurements of Ca homeostasis, or Hct. In a prospective

TABLE 4.
Serum Al Levels ($\mu\text{g/L}$) Mean \pm SD in Patients
Undergoing Peritoneal Dialysis

Modality*	Number	Serum Al	SD	Reference
IPD	23	58	31	253
IPD	10	138	70	254
CAPD	51	53	41	45
CAPD	12	54	NA	255
CAPD	22	42	NA	205
CAPD	18	31	14	47
CAPD	29	47	8	41
CAPD	16	55	11	48
PD + hemodialysis	37	180	121	49
PD†	5	88	55	43

*IPD = intermittent peritoneal dialysis; CAPD = continuous ambulatory peritoneal dialysis; PD = peritoneal dialysis.

†Type of PD not specified.

TABLE 5.
Serum Aluminum in Children on CAPD (Dialysate
Al $< 5.0 \mu\text{g/L}$)*†

Duration of CAPD (months)	Serum Aluminum ($\mu\text{g/L}$)	Aluminum Intake (mg/kg/day)
7.9 \pm 2.1	55.2 \pm 11.4	98 \pm 20
16.6 \pm 2.3	59.8 \pm 10.4	104 \pm 32

*Adapted from Gruskin AB, Polinsky MS, Lerner GR, et al: Aluminum intoxication in children, in Strauss J (ed): *Renal-GU Disorders: Progression, Replacement Therapy, Growth—Current Concepts in Diagnosis and Management*. Miami, University of Miami Press, 1987, pp 223–232. Salusky IB, Coburn JW, Paunier L, et al: Role of aluminum hydroxide in raising serum aluminum levels in children undergoing continuous ambulatory peritoneal dialysis. *J Pediatr* 1984; 717–720.

†In normal children, serum aluminum = $8.2 \pm 1.1 \mu\text{g/L}$ and there is no daily aluminum intake.

TABLE 6.
Relationship of Serum Aluminum to Dialysis Modality
Phosphate Intake and Midrange PTH*†

	Peritoneal	Hemodialysis	P value
Serum aluminum	180.8 +/- 121.1	278.6 +/- 233.1	< 0.05
Phosphate intake	31.6 +/- 21.8	46.0 +/- 34.1	<0.05
iPTH	674.7 +/- 845.2	976.7 +/- 959	0.16

*Adapted from Polinsky MS, Kaiser BA, Root AW, et al: The effects of phosphate binder dose on serum aluminum levels in pediatric maintenance dialysis patients. *Pediatr Res* 1986; 20:456A.

†Normal values: Al = 10.4 +/- 7 µg/L; iPTH < 250 pg/ml.

study involving 87 adult patients on maintenance hemodialysis, those with serum levels greater than 190 µg/L developed clinical Al toxicity while those with Al levels less than 135 µg/L did not.⁴² As previously mentioned, children may experience Al toxicity at lower serum Al levels than adults.

In dialysis patients with ABD, serum Al levels tend to be higher than in renal patients not yet on dialysis with other forms of renal osteodystrophy.⁵¹ Considerable overlap exists between serum Al levels and various forms of renal osteodystrophy.⁵²

Serum Al levels increase after DFO infusion in both adults and children with increased tissue stores of Al, particularly Al in bones. DFO effectively removes circulating ionized Al from blood, reduces the ratio of free to bound Al in blood, and allows additional Al to be transferred from bone to blood. The DFO infusion test is increasingly being used to evaluate the body burden of Al.⁵³ Its use is further discussed in the section on ABD.

Periodic monitoring of the blood Al concentration (two to four times per year) is indicated in children with advanced renal failure and in those undergoing maintenance dialysis, especially if they are receiving ACPBs.

Aluminum Intoxication in Patients With Renal Failure

Central Nervous System Effects of Aluminum

Experimental Studies

Al alters CNS and peripheral nervous system function in animals. Motor and behavioral changes develop in rabbits and cats 1 to 2 weeks following

infusion of Al into brain. Deficits in motor coordination are followed by a progressive encephalopathy, apathy, incapacitation, focal seizures, and then generalized seizures and death.⁵⁴ These changes are accompanied by the development of an abnormal EEG and evoked potentials.⁵⁵ Effects on short-term memory, conditioning, and retention can be demonstrated.⁵⁶ At autopsy, neurofibrillary tangles are present in the dendrites and stroma of neurons and are absent in axons and glia. A single subcutaneous dose of DFO 2 hours after intracisternal Al administration followed by a dose of DFO every 3 days failed to prevent encephalopathy in three of four rabbits.⁵⁷

Species differences exist. The rhesus monkey takes more than a year to develop tangles. Mice and rats do not develop neurofibrillary tangles. Neither species develops encephalopathy at Al doses tenfold higher than those causing encephalopathy in rabbits and cats.⁵⁸ Al-susceptible animals bind 80% of Al on to the chromatin of glia and neurons within 1 hour after an intracranial injection; resistant animals bind only 20% to 30%.⁵⁹

Older animals develop CNS and behavioral changes at lower doses of Al. Neurofibrillary tangles develop in the perinatal period and in adults at similar doses of Al.⁶⁰ In the neonatally treated animals, neither neurologic nor behavioral changes occurred at twice the adult dose of Al; however, approximately 75% of the younger animals developed neurologic impairment at three times the adult dose.⁶¹ In cats and rabbits, neurologic dysfunction occurs when the Al content of brain exceeds control levels by a factor of three to six. It appears that Al levels in the adult human brain need to increase 10 to 20 times before neurologic signs develop.⁵⁶

As for peripheral nerve function, in rabbits made Al toxic by daily subcutaneous injections of 60 mg/kg/day for 30 days, peripheral neuropathy develops.^{62, 63} Nerve conduction velocity studies show a primary axonopathy. Serial electromyographic studies initially reveal acute denervation with fibrillations, bizarre high-frequency discharges, and subsequently, complex high-amplitude motor unit potentials. Histologic study shows denervation and group atrophy in muscle and nonspecific changes in peripheral muscle. In vitro studies show that Al partially blocks acetylcholine-evoked contractions of human smooth muscle⁶⁴ and blocks calcium uptake in rat colon longitudinal smooth muscle.⁶⁵

Pathophysiology

Al affects a number of transport and enzyme systems in the CNS. Al is inhibited by calmodulin, which is involved with a large number of transport systems, binding proteins, and receptors in the CNS.^{28, 66} Al decreases Ca and Ca-Mg ATPase activity.²⁷ This sequence of alterations may explain the high Ca levels in brain tissue reported in patients with Al encephalopathy.⁶⁷ Al inhibits systems involved in the uptake of choline, glutamate, γ -aminobutyric acid, and serotonin. Al can displace copper, essential for brain cytochrome oxidase activity, from some of its metalloenzymes⁶⁸ and ferroxidase (ceruloplasmin).

In animal studies using Al ion concentrations 100 times that found in patients with dialysis dementia, no effects were found on brain mitochondrial pH, O₂ consumption, or stimulation with adenosine diphosphate.⁶⁹ Polarographic studies, however, of isolated rabbit brain mitochondria obtained after systemic injections of Al have shown 20% and 50% decreases in oxidative phosphorylation in cerebrum and cerebellum, respectively.⁶³

Clinical Features

What is now known as Al encephalopathy was originally described as the dialysis encephalopathy syndrome (DES); the terms are now used interchangeably. Originally it was thought that the syndrome occurred only in patients undergoing hemodialysis. Adults with DES follow a typical pattern.^{70, 71} The initial presentation is a mild speech disturbance characterized by stammering and stuttering, often made worse by hemodialysis. Next, changes in personality and directional disorientation occur. The speech disturbance becomes worse with periods of dyspraxia, hesitancy, and muteness. Anomia, hypofluency, muscle fatigue, myoclonus, motor apraxia, ataxia, and asterixis develop. Focal, multifocal, or grand mal seizures; lethargy; depression; and altered mood typically occur. Patients often develop auditory and visual hallucinations. Paranoia and suicidal tendencies may be seen. Eventually the patient becomes immobile, obtunded, and nonresponsive; death occurs 6 to 9 months after the onset of symptoms. A typical EEG pattern evolves with abnormalities preceding clinical symptoms by 4 to 6 months. It is characterized by multifocal bursts of slow (delta) and spike activity with normal background activity. Computed tomography (CT) scans are usually normal but may show cortical atrophy. In retrospect, it took about 2.5 years of dialysis with untreated water for patients to be at risk and 4.5 to 6.5 years for them to develop clinical symptoms.⁷¹ Speech screening tests have been used to detect patients with early DES.⁷²

The world-wide occurrence of Al encephalopathy in children was established by a survey of 96 pediatric nephrology programs. The 63.5% responding were caring for 1,616 children.⁷³ Fourteen of 61 centers (five foreign, nine American) caring for 728 children reported on 24 affected children. Most children had congenital genitourinary disorders (73.8%) and all had been receiving ACPBs. Reported symptomatology included personality changes, 92%; dementia, 85.7%; regression of developmental landmarks, 69.2%; speech impairment, 78.6%; and seizures, 64.3%. Dialysis had not been started in nine children; thus, it was clearly established that encephalopathy occurs independently of hemodialysis. Additional children as well as adults not yet started on dialysis have been reported to develop encephalopathy as well as elevated serum and bone Al levels.⁷⁴⁻⁷⁶ Adults and children undergoing peritoneal dialysis⁷⁷ or Al-free hemodialysis can develop Al encephalopathy. The source of Al is oral ACPBs.⁷⁸

The typical neurologic features of DES are somewhat different in chil-

dren. Many affected children develop encephalopathy during the phase of chronic renal insufficiency prior to their requiring dialysis. Encephalopathy has occurred at a GFR of 5 to 25 ml/min 1.73 sq m.^{3, 79} Affected children often display cerebellar signs, hypotonia, muscle weakness, mental retardation, regression of developmental milestones, speech disturbance, myoclonus, minor and major seizures, coma, and death. Nystagmus, athetoid movements, and twitching tongue movements have been seen. Head circumference is within normal limits in most affected children.^{4, 80} The encephalopathy of progressive renal failure in children has been described as evolving through three stages of neurologic change (Table 7).⁴ A bulging anterior fontanel has also been described.⁸¹ Regression of neurologic function was demonstrated in one child in whom formal psychologic exami-

TABLE 7.
Progression of Neurologic Changes in
Children Developing the
Encephalopathy of Progressive Renal
Failure*

Stage 1

Ataxia, mild
 Dysmetria
 Failure to develop new motor skills
 Hyperreflexia
 Plantar extensor signs
 Tremors

Stage 2

Ataxia, marked
 Hypotonia
 Myoclonic seizures, extremities and facial
 Regression in cognitive and motor function
 Saccadic ocular movements

Stage 3

Absent response to auditory and visual stimuli
 Absent swallowing with intact gag reflex
 Hypotonia, marked
 Hyperreflexia
 Myoclonus, generalized
 No volitional movements

*Adapted from Foley CM, Polinsky MS, Gruskin AB, et al: Encephalopathy in infants and children with chronic renal disease. *Arch Neurol* 1981; 38:656-658.

nation (Bailey and Denver development testing) was normal at age 2.⁸² At age 6, IQ was 57; three other tests revealed multiple deficits of learning, processing, language, and memory. Multiple neurologic deficits were also demonstrated. CT scans in some affected children show cortical atrophy. The relationship between Al and atrophy, however, is unclear.

In one prospectively followed group of 14 children less than 5 years old with chronic renal failure (CRF) not requiring dialysis, most demonstrated motor delay in the absence of microcephaly or severe retardation.⁴⁴ All, however, developed behavioral and/or cognitive problems that require special education. The majority received ACPBs and had elevated serum Al levels. Additional data are still needed to ascertain whether Al can affect cognitive function without simultaneously causing motor signs and/or symptoms.

Infants with renal failure thought not to be ingesting significant quantities of Al may experience developmental delay and CNS problems. In the absence of ingesting ACPBs, 20 of 23 infants with CRF were reported to have developmental delay, reduced rates of growth in head circumference, seizures and abnormal EEG patterns.⁸³ Whether these children had received either formula or intravenous drugs that contained Al was not detailed and measurements of serum Al were not made. In another series of five children with CRF diagnosed prior to 5 months of age followed prospectively, the standard deviation score for head circumference fell progressively during the first 12 to 18 months of life and then stabilized.⁸⁴ Head circumference remained within normal limits and was less severely affected than was linear growth. Developmental indices were within normal limits in four of the five. One infant received ACPBs; four did not. Serum Al concentrations were measured in two infants and was elevated in one. Developmental delay has also been observed in infants treated with continuous cycle peritoneal dialysis never given ACPBs. Even though these groups of infants did not receive ACPBs, it is possible that they had been exposed to Al from other sources and that their neurologic deficits were in part related to Al. Conversely, other factors, not yet identified, may be involved. Also, a case of encephalopathy secondary to an industrial exposure of Al powder has been described.⁸⁵

Al encephalopathy occurs in two settings, epidemic and sporadic. Some hemodialysis centers experienced a large number of cases of encephalopathy while others did not. Epidemiologic surveys established that involved centers had used dialysate with a high Al content.^{86, 87} That ABD was part of the Al intoxication complex was initially recognized in patients developing encephalopathy.⁸⁸ A case-control study in which patients were prescribed similar amounts of elemental Al and vitamin D found that those with encephalopathy had more fractures, less hyperparathyroid bone disease, a higher blood urea nitrogen, and a lower hemoglobin content.⁸⁹ The water content of Al used in these centers had Al concentrations in the range of 100 to 300 $\mu\text{g/L}$ resulting in a net transfer of Al to patients of 2 to 3 mg per dialysis.⁹⁰ In centers with a large number of DES cases, re-

removal of Al from water used to prepare dialysate led to dramatic reductions in new cases.^{91, 92} It was eventually realized that the oral Al load—in addition to that contained in tap water used to prepare dialysate—had pathophysiologic significance and accounted for many of the sporadic cases of Al encephalopathy.

Acute deterioration in CNS function following parathyroidectomy has been reported in Al-intoxicated adults⁹³ and in one child. Others have not observed an increase in Al toxicity after parathyroidectomy in children.⁹⁴ Postoperative immobilization may allow bone sequestered Al to move into the blood stream and to be redeposited in brain. It also has been suggested that brain Al accumulation increases after bone surfaces are saturated.⁹⁵

Aluminum Metabolism and Brain Aluminum

In patients dying from dialysis dementia, Al levels are elevated in bone, heart, liver, muscle, and spleen as well as brain.⁹⁶ The body burden of Al in patients with DES has been estimated to be approximately 1.0 gm at autopsy⁹⁷ and 1.1 gm by whole body in vivo neutron activation analysis.⁹⁸ In general, there is a progressive rise in brain Al content as patients experience renal insufficiency, dialysis without encephalopathy, and finally dialysis with encephalopathy. Mean brain Al content in normal brain is usually less than 4.0 mg/kg. Mean brain Al concentrations in nondemented adult dialysis patients ranges from 3.8 to 8.5 mg/kg,⁹⁹ while brain Al concentrations in demented patients range from 12.4 to 33.0 mg/kg.^{2, 100–102} Values, however, for whole brain Al content overlap in dialysis patients with and without dialysis dementia. Asymptomatic patients with CRF often have higher concentrations of brain Al than patients on dialysis. Brain Al content is also elevated in patients with acute renal failure.¹⁰³

Gray matter Al content is significantly higher than that of white matter in affected adults.^{2, 59, 102} A positive correlation between months on dialysis and gray matter Al content is found in patients with DES.^{2, 102} Al in affected patients is located in the cytoplasm of cells.

The neurofibrillary tangles characteristic of Al toxicity in the experimental animal are not seen in human autopsy material. However, such tangles can be experimentally produced in human neocortical cells.⁵⁶ In humans, Al is found in oligodendroglia and astrocytes but not neurons.⁶⁶

Elevated brain Ca¹⁰⁴ and Mn¹⁰⁵ levels were thought to contribute to the development of Al encephalopathy. Elevated brain Mn has been reported in white matter of affected patients who also had elevated brain Al.¹⁰⁵ Two findings, however, argue against brain Ca playing a primary role in causing encephalopathic symptomatology. One is that brain Ca content is similar in dialysis patients with and without DES.⁶⁸ The second is that a syndrome similar to DES—with the exception of a similar EEG—has been attributed to hypercalcemia.¹⁰⁶

Two infants with CRF have been reported to have high levels of brain Al, 6.4 and 47 $\mu\text{g/gm}$, in the absence of ingesting ACPBs, receiving Al-

containing intravenous medications, or being exposed to AL-containing dialysate.³³ Bone Al content in these infants was normal. The source of Al was attributed to proprietary infant formula. The brain Al content of a 2.2-year-old child with encephalopathy was similar to that of a 20-year-old with CRF but without encephalopathy.⁸⁰ Both had values higher than those obtained in three control children less than 2 years old; the second had an elevated serum Al level. Both affected patients had elevated serum Ca and PTH levels. In one, the brain Ca was marginally elevated above the highest control value obtained. In another study comparing children to adults, brain Al content in both gray (80 $\mu\text{g/gm}$) and white (47 $\mu\text{g/gm}$) matter in a child with bilateral renal hypoplasia dying at 8 years of age after receiving 43 mg Al/kg/day between the ages of 2 and 6 exceeded values reported in adults with DES.¹⁰⁷

In a review summarizing 28 cases of progressive encephalopathy in children with CRF from infancy, including the aforementioned cases, evidence for Al toxicity existed in seven. Twenty-six received conservative management until the onset of neurologic symptoms and most had their onset prior to 2 years of age.⁸⁴

In patients with encephalopathy, cerebrospinal fluid (CSF) Al levels are not usually elevated, but some exceptions exist. In four patients with DES, CSF Al levels were approximately 40 to 68 $\mu\text{g/L}$ compared with values of 15 to 20 $\mu\text{g/L}$ in two patients on dialysis; CSF Al concentrations are usually less than 10 $\mu\text{g/L}$ in controls.⁹⁷ In three of the five children originally described as having Al-related encephalopathy, CSF Al levels were normal when measured many months after stopping ACPBs. One child still had a serum Al greater than 150 $\mu\text{g/L}$.⁴⁹ A five-month-old child with renal failure and encephalopathy had a CSF Al concentration of 14 $\mu\text{g/L}$.⁸¹ In another affected child, serum and CSF Al levels were 178.5 and 3.70 $\mu\text{g/L}$, respectively. Inflamed meninges may allow Al to more readily enter the CSF. CSF Al was elevated in an adolescent with neurologic deficits and *listeria* meningitis. In affected children, CSF concentrations of copper, norepinephrine, dopamine, and immunoglobulins are normal.⁴

Pathologic examinations of brains are limited in children dying with encephalopathy. Hypertrophy of cortical astrocytes, severe neuronal loss, and spongy degeneration in a small brain with dilated ventricles and widened sulci were found postmortem in a child dying at age 6 after developing neurologic symptoms 2 years previously.⁴ Autopsy examinations of adults dying from DES reveal glial proliferation, spongy degeneration, cytoplasmic and nuclear shrinkage, nuclear hyperchromicity, and loss of Nissl substance of the outer granular layer of the cerebellum.⁷⁰ Viral cultures and/or brain tissue from affected patients injected into primates, except for a single report, have not caused disease.¹⁰³

Therapy For Aluminum Encephalopathy

Improvement in neurologic function occurs following cessation of oral Al intake in patients with mild encephalopathy.^{108, 109} Therapy is usually less

effective for more advanced encephalopathy. Patients on dialysis have been reported to improve after being switched from Al-containing to Al-free dialysate,^{93, 110} with worsening occurring after reinstitution of ACPBs.¹¹¹

Clonazepam improved seizure control, but not myoclonus, in some children. Phenytoin, phenobarbital, and diazepam have not improved seizure control in some children,⁴ but diazepam may be helpful in treating myoclonus and seizures early in the course of DES. Improvement, no change, and worsening have been reported after parathyroidectomy.^{104, 111} Parathyroidectomy has failed to improve encephalopathy in affected children.

After renal transplantation, some adults with DES improve^{112, 113} while others deteriorate more quickly.¹¹¹ Acute neurologic deterioration thought to be related to Al has been observed in a few children within a few weeks after renal transplantation.¹¹⁴ Delayed graft function can be associated with marked increases in serum Al levels while good graft function increases Al excretion.¹¹⁵ In one report, serum Al levels fell steadily in post-transplant patients who had prompt and good graft function.¹¹⁶ Brain Al content has been found to be elevated 4 years after transplantation.¹⁰⁸ Six of 15 English children transplanted before 5 years of age, possibly exposed to ACPBs, and systematically followed were reported to be moderately to severely retarded.⁴⁴ Possible risk factors were time on dialysis prior to transplant and the accumulated Al load.

DFO has been used to successfully treat Al encephalopathy in adults; however, therapy is not universally effective.¹¹⁷⁻¹²⁰ DFO infused over 2 hours weekly (40 to 80 mg/kg) or biweekly to six adults (in two, DFO therapy was reduced and given with each dialysis) resulted in striking clinical improvement in five. Serial EEGs returned to normal in one, improved significantly in two, and remained unchanged in two despite clinical improvement.

Reversal of major neurologic deficits has also been described in children treated with DFO.¹²¹ In one child, 6 weeks of DFO (40 mg/kg) instilled once weekly in the overnight 250-ml CAPD bag led to marked improvement in neurologic function and a concurrent decrease in serum Al.⁸¹ Abnormal EEG tracings returned to normal.

DFO therapy, however, can also worsen neurologic symptomatology. Its neurologic effects may be dose dependent.¹²² Increased speech impairment and increased seizure activity can follow DFO infusion in children¹¹⁴ and adults. In a 2.5-year-old child, staring spells were associated with intraperitoneal DFO administration. A concomitant increase of plasma Al to 1,959 $\mu\text{g/L}$ occurred.¹²³ In patients who develop worsening symptomatology after DFO administration, lowering the dose of DFO and more frequent monitoring of blood Al levels are indicated. The negative influence of DFO on neurologic function is attributed to its mobilizing of bone Al, increasing blood levels and exposing the CNS to additional Al. Plasma Al concentrations should be less than 1,000 $\mu\text{g/L}$ during treatment with DFO to avoid CNS complications.⁹⁴

Although additional data are needed, infants with encephalopathy treated with DFO may experience less improvement in neurologic function than adults. Neurologic damage may be irreversible if it is sustained during infancy secondary to Al intoxication.¹²³ It is not known if DFO therapy actually lowers brain Al content.

Aluminum Bone Disease

The most complete data relative to Al toxicity are those defining the effect of Al on bone. ABD was first reported in 1969 as "fracturing osteomalacia" in hemodialysis patients from Newcastle Upon Tyne, England.¹²⁴ The reported incidence of ABD in the United States ranges from 3%–4% to 30%–75% in patients with musculoskeletal symptoms.^{125–127} The features of ABD include weakness, myopathy, bone pain, pathologic fractures, and unresponsiveness to vitamin D metabolites. The weakness predominately involves proximal muscles and the fractures often involve ribs and the small bones of the foot.

Experimental Studies

Experimental studies show a relationship between Al intake, Al deposition in bone, and impaired mineralization. With Al administration, histologic findings in bone—similar to those in dialyzed patients—can be produced in dogs²² and rats¹²⁸ with and without reduced renal function. The effect is dose,¹²⁹ time,¹³⁰ and route dependent.¹³¹ In normal rats given intraperitoneal Al daily for 48 to 85 days, bone X-ray films and histologic examination after 52 days was unremarkable despite a bone Al content similar to that found in hemodialyzed patients.¹³⁰ Weight gain was impaired after 2 to 3 weeks and returned to normal when Al was discontinued. After 63 days, evidence of osteomalacic bone disease was found and persisted after Al had been stopped for 49 days. When compared with normal rats, linear growth rates were diminished in the Al-treated rats.

In normal and 5/6 nephrectomized rats given a combination of oral 1% to 2% AlCl_3 or 3.0 ml $\text{Al}(\text{OH})_3$ (150 mg/kg elemental Al) and intraperitoneal or subcutaneous injections of Al, all died within 3 to 8 days after developing periorbital bleeding, anorexia, and lethargy.¹³¹ Postmortem studies showed conjunctival epithelial ulceration and an elevated Al content in bone, liver, muscle, heart, and brain. In rats given intraperitoneal Al, Al content was highest in liver; in those receiving only oral Al, Al content was highest in bone. In vitro oxygen consumption by the liver fell 25%.

Osteomalacia developed in normal rats given 1.0 mg of Al daily for periods as long as 120 days; osteomalacia did not develop in rats untreated or given 0.1 mg/day.¹²⁹ The combination of chronic renal failure and Al toxicity was associated with more severe osteomalacia, and CRF alone did not lead to osteomalacia. Al toxicity resulted in marked prolon-

gation of the mineralization lag time and reduced bone formation. These data suggest that Al intoxication in growing animals results in reduced rates of linear growth.

Reductions in bone formation rates may be a direct effect of Al. When compared with controls, thyroparathyroidectomy in rats followed by Al injections (2 mg/day) for 42 days was associated with greater impairment of osteoid production and similar bone formation rates.¹³² Bone Al content was similar in Al-loaded controls and Al-loaded thyroparathyroidectomized rats. Thus, low PTH levels may contribute to the pathogenesis of aplastic bone disease, but reductions in bone formation rates in Al-loaded rats are not mediated by PTH.

Al deposition in osteomalacic bone may be an epiphenomenon rather than the cause of impaired bone metabolism. In growing dogs made vitamin D deficient and given Al, mineralization subsequently occurred with vitamin D therapy.¹³³ Bone histologic patterns returned to normal and Al was demonstrated in the cement lines of healed dog bone indicating that mineralization occurred at sites of Al deposition. Furthermore, the multiple factors operating on bone during growth may continue to do so despite ABD. Suggestions for the failure to observe ABD in normal controls despite their accumulating Al in their bones compared with the other experimental group included the amount of Al given, the duration over which it was given, and the absence of concomitant renal disease. The differences between the osteomalacia of Al accumulation and vitamin D deficiency as well as other experimental studies continues to support a direct role for Al in causing bone disease.

Al may interfere with calcium flux,¹³⁴ is found in mitochondria of osteoblasts at the osteoid calcification junction,¹³⁵ and complexes with citrate.¹³⁶ Al can deposit in articular tissue,¹³⁷ inhibit the precipitation of calcium phosphate,¹³⁸ inhibit radiocalcium uptake in embryonic chick bone,¹³⁹ and inhibit formation of hydroxyapatite crystals.^{140,141} In vitro collagen synthesis is impaired by osteoblasts exposed to high concentrations of Al.¹⁴²

Studies of Al on isolated cultured osteoblastic and osteoclastic cells prepared from neonatal mouse calvaria reveal a biphasic effect.¹⁴³ Lower concentrations of Al ($<10^{-6}$ M) stimulated collagen and DNA synthesis, ornithine decarboxylase, and alkaline phosphatase in osteoblastic-like cells. Higher concentrations of Al ($>10^{-6}$ M) had an opposite effect. Al inhibited the stimulatory effect of PTH on cyclic adenosine monophosphate content and altered the $1,25(\text{OH})_2\text{D}_3$ effects on ornithine decarboxylase activity of the osteoblastic-like cells. These effects provide evidence that Al may influence bone remodeling by interfering with the normal actions of PTH and $1,25(\text{OH})_2\text{D}_3$ on bone cells. Insofar as the effects of $1,25(\text{OH})_2\text{D}_3$ on bone are similar to those in the intestine, the resistance to $1,25(\text{OH})_2\text{D}_3$ in Al-treated rats is at a site distant to its receptor site.¹⁴⁴

Aluminum Effects on Bone in Patients With Renal Failure

Al affects bone mineralization in at least three ways. It alters mineralization at the osteoid calcification boundary; it alters osteoblast function; and it influences PTH secretion.

A number of histomorphometric techniques are available to quantify features of normal and diseased bone.⁹⁵ Normal trabecular bone is 3% unmineralized and 97% mineralized. Osteoid matrix covers 25% of the bone surface and is lined by osteoblasts. Osteoclasts cover 4% of the trabecular surface of bone.¹⁴⁵

The types of renal osteodystrophy include pure osteomalacia, pure osteitis fibrosa, mixed bone disease, and aplastic bone disease.¹⁴⁶ Aplastic bone disease is thought to be the purest form of ABD. The morphologic features of renal osteodystrophy are summarized in Table 8.¹⁴⁶ The histologic classification of ABD, however, remains controversial. Histologic criteria suggestive of pure ABD include an increased osteoid volume that is patchy in distribution, reduced numbers of osteoblasts and osteoclasts, minimal or absent fibrosis, and a reduced rate of mineralization measured by the double tetracycline labeling technique.¹⁴⁷ Some believe that one criterion for diagnosing functional ABD should be a reduced bone formation rate in addition to demonstrating Al accumulation. Additional criteria include the demonstration of Al at the osteoid mineralization boundary and Al in excess of 10 $\mu\text{g/gm}$ wet bone weight.¹⁴⁸ The aluminon (aurine tricarboxylic acid) stain is Al specific and gives a bright red color.¹⁴⁹ Data defining the relationship between the degree of Al staining and the measured Al content of bone have not always been consistent.^{150, 151} Iron accumulation has been described together with Al at the mineralization front.¹⁵² Some have suggested that lead, silicon, and sulfur may also accumulate. Proof of sulfur accumulation is lacking.

In patients undergoing dialysis, aplastic bone disease may exist singly or in combination with other forms of renal osteodystrophy. Some have found no differences in bone Al content between patients with osteomalacia and osteitis fibrosa¹⁵³; others report that the bone Al content is increased in patients with osteomalacia.¹⁵⁴ In such patients, however, bone Al content is usually less than it is in patients with aplastic bone disease.¹²⁵

The "gold" standard for diagnosing ABD is a bone biopsy. While a number of attempts have been made to diagnose ABD noninvasively by using the DFO infusion test, not all patients with biopsy-proven ABD experience increased serum Al levels following DFO infusion.^{155, 156}

Correlations between histopathologic indices, bone Al content, and stainable bone Al were studied in 55 patients undergoing hemodialysis.¹⁵¹ Bone Al content correlated with lamellar osteoid. Stainable Al correlated with bone formation rate, mineral apposition rate, and cellular measurements of bone formation and resorption. It was concluded that Al deposition at the mineralization front results in other histologic findings and that Al in trabecular bone is of minimal significance. In patients undergoing

TABLE 8.
Renal Osteodystrophy in Children*

Normal	Normal osteoid, no resorption, and normal mineralization
Pure osteitis fibrosa	Increased bone resorption (fibrotic surface [%] ≥ 1 and/or osteoclast number [per sq mm] >2 SD above the mean) and increased mineralization (mineralization front [%] >2 SD above the mean)
Pure osteomalacia	Increased osteoid (osteoid volume [%] >2 SD above the mean), no resorption (osteoclast number and fibrotic surface normal), and decreased mineralization (mineralization front [%] >2 SD below the mean)
Mixed bone disease	Increased osteoid (osteoid volume [%] >2 SD above the mean and/or osteoid surface [%] >2 SD above the mean) and increased resorption (fibrotic surface [%] ≥ 1 and/or osteoclast number [per sq mm] >2 SD below the mean)
Aplastic bone disease	Normal to decreased osteoid (osteoid volume [%] at or below the mean and/or osteoid surface [%] at or below the mean) with decreased osteoblastic osteoid (osteoblastic osteoid surface [%] >2 SD below the mean), no resorption, and decreased mineralization (mineralization front [%] >2 SD below the mean)

*Adapted from Chesney RW, Mehls O, Anast CS, et al: Renal osteodystrophy in children: The role of vitamin D, phosphorus, and parathyroid hormone. *Am J Kidney Dis* 1986; 7(4):275-284.

dialysis, stainable Al rather than the quantity of Al in bone may be more reflective of the nature of the histopathologic changes in bones.¹⁵⁰ Even though mean serum Al levels were significantly higher in patients with (107 $\mu\text{g/L}$) than without (31 $\mu\text{g/L}$) stainable bone Al, considerable overlap existed. Repeat bone biopsy specimens, obtained 11 to 16 months apart, demonstrated a decrease in cancellous bone mass and osteoid-osteoblast interface and no change in osteoclast interface. These findings suggest that Al can cause osteopenia, a loss of bone mass. Al staining along cement lines surrounded by calcified bone suggest that healing of ABD does occur and that the negative effects of Al on bone mineralization can be overcome.

In children, increases in bone Al content generally exist in conjunction

with high blood levels of Al.^{43, 45} In one child with renal insufficiency begun at 5 months of age on a dose of elemental Al that varied from 31 to 108 mg/kg/day, the serum Al concentration at 31 months of age was 334 $\mu\text{g/L}$.⁷⁴ Bone Al content was 156 mg/kg of dry bone compared with bone Al concentrations that were 3.0 and 5.5 mg/kg of dry bone in two control children. Another 7.5-year-old child with Al encephalopathy had serum and bone Al concentrations of 220 $\mu\text{g/L}$ and 165 mg/kg, respectively.⁸² This child received $\text{Al}(\text{OH})_3$ 5.0 ml three times a day for at least 4 years and possibly 5.5 years before the bone biopsy. Children with similar plasma Al levels can have also marked variation in bone Al content. Bone Al content was increased in a 6-month-old infant in the absence of any clinical or biochemical evidence (details not given) of ABD.⁴⁴

Clinical Features

ABD occurs in two distinct clinical settings.⁵¹ The first is the sporadic form of Al toxicity that usually occurs in patients taking ACPBs and develops in patients being dialyzed with appropriately treated water. Typical clinical characteristics include myopathy, multiple bone fractures, a tendency to hypercalcemia, elevated alkaline phosphatase levels, and iPTH levels lower than those usually seen in dialysis patients. The second form is that of epidemic Al toxicity and is associated with exposure to high dialysate Al concentrations. Clinical features include microcytic anemia, a high incidence of encephalopathy (41.0% vs. 6.0%), and ABD. Blood Al concentrations are usually above 75 to 100 $\mu\text{g/L}$; serum levels are 50% higher in the epidemic compared to the sporadic form. Plasma Al concentrations in patients with microcytic anemia are apparently higher when patients experience epidemic Al intoxication. ABD may also occur in otherwise asymptomatic patients on dialysis.¹⁵⁷

Serum Ca levels are usually normal or minimally elevated.¹⁵⁸ Hypercalcemia may be spontaneous or associated with oral calcium supplements, transfer of calcium from dialysate to patient,¹⁵⁹ or the ingestion of vitamin D metabolites. Alkaline phosphatase concentrations in patients with ABD secondary to ACPB ingestion are usually elevated; values in those whose source of Al is dialysate tend to be normal. In one study, alkaline phosphatase levels were similar in dialysis patients with ABD and osteitis fibrosa.⁵¹ Serum phosphate levels are usually normal or high and iPTH activity normal to minimally elevated. ABD is often misdiagnosed as hyperparathyroidism in patients with hypercalcemia and minimal and/or normal iPTH levels. Serum levels of 25(OH)-vitamin D are normal and exclude classical nutritional vitamin D deficiency.¹⁶⁰ Serum levels of 1,25(OH)₂D₃ are low as expected in patients with ESRD.¹⁶¹

Radiologic features that, however, are not diagnostic of ABD include osteopenia and widening of the physes and fraying of the metaphyses of long bones in the absence of the typical subperiosteal erosions associated with hyperparathyroidism. Looser's zones can be seen. Bone films in patients with Al-related osteomalacia may be interpreted as consistent with

hyperparathyroidism because they display pseudosubperiosteal resorption. Resorption occurs in patients with Al toxicity when osteoblasts lay down excessive osteoid that fails to mineralize. The subperiosteal resorption persists when iPTH levels fall in patients with ABD.¹⁶² Bone scintiscans in patients with Al toxicity may reveal a reduced uptake of radiolabeled bisphosphonate when compared with dialysis patients with osteitis fibrosa¹⁶³ and pseudofractures, which are not seen with conventional radiographs.^{164, 165} Data on bone scintiscans remain controversial.

Healing of ABD in two children was associated with the radiographic finding of the development of a "bone within a bone" followed by calcification of the radiolucent area.¹⁶⁶ The recalcification picture began at the most recently formed osteoid areas with the epiphysis and metaphysis leaving a radiolucent area between previously and newly calcified bone. Craniosynostosis requiring craniectomy occurred in one child recovering from ABD.

Al deposition at the calcification front between mineralized bone and osteoid was reported in a 1.5-year-old child with serum Al levels ranging from 174 to 208 $\mu\text{g/L}$.⁴⁸ In three infants, ages 4, 9, and 23 months with serum Al levels of 595.0, 344.5, and 385.5 $\mu\text{g/L}$ secondary to $\text{Al}(\text{OH})_3$ ingestion, the characteristic histomorphometrical findings of ABD were found.⁴⁵ Bone Al occupied 2.95, 7.21, and 1.38 mm of Al per sq mm of tissue area. Two infants had pure low bone-turnover osteomalacia; the third had a combination of osteomalacia and mild hyperparathyroidism.

In 14 children with osteitis fibrosa on CAPD, mean serum Al was $33 \pm 18 \mu\text{g/L}$, bone Al was $39 \pm 31 \text{ mg/kg}$ (normal $< 7.0 \text{ mg/kg}$), and iPTH was $454 \pm 520 \mu\text{Eq/ml}$.¹⁶⁷ Bone formation rate and reabsorbing surface were increased and correlated positively with iPTH values. Bone Al was inversely correlated with iPTH values and osteoid volume, and bone Al measurements had no relationship to bone formation rates. It was concluded that either bone accumulation of Al modifies PTH secretion or that the PTH being secreted may modify Al deposition in bone in children with secondary hyperparathyroidism. Others, however, have reported that serum Al levels and PTH levels are not positively correlated in children with ABD.⁴⁵

Since ABD is characterized by a reduced rate of formation of new bone, it should affect growing children by reducing the growth rate of bone to levels lower than that associated with CRF per se. Children with ABD generally do have stunted growth patterns, but comparative growth data on children with and without ABD are not available. The impact of successful therapy of ABD on growth rates in affected children remains unknown.

Aluminum Effects on Parathyroid Gland Function

Al alters parathyroid gland function, and three mechanisms have been suggested as causes of PTH suppression in Al-intoxicated patients: direct suppression of PTH secretion by AL,¹⁶⁸ Al accumulation within the parathyroid gland,¹⁶⁹ and hypercalcemia. Bilateral nephrectomy may worsen

ABD. The mechanism may be secondary to calcitonin-mediated suppression of PTH secretion.^{125, 170}

Al accumulates at higher concentrations in the parathyroid gland than it does in muscle or the thyroid gland.¹⁶⁹ Al inhibits the secretion of PTH from parathyroid slices¹⁷¹ or cells.¹⁶⁸ Al added to the bathing media of normal parathyroid glands decreases ionized Ca and adenylate cyclase activity¹⁷² and it also interferes with the allosteric metal activating sites.¹⁷³ Inverse relationships between bone Al content and iPTH¹⁶³ and parathyroid gland weight¹⁷⁴ have been reported. Using isolated parathyroid gland cell preparations, studies of the effect of Al on PTH synthesis have revealed a dose-dependent effect of Al, both an Al-sensitive and Al-insensitive effect, and a persisting effect on changes in calcium concentration.¹⁷⁵ Al irreversibly destroyed some cells while other cells remained intact.

PTH responsivity to hypocalcemia is reduced in patients with ABD.¹⁷⁶ Part of the confusion relating to PTH function and Al can be explained by conclusions drawn when C-terminal PTH levels rather than N-terminal levels are measured. In one study, N-terminal concentrations were normal in patients with ABD and lower than normal in patients with osteitis fibrosa. C-terminal PTH levels were similar in both groups.¹⁷⁷ PTH serum concentrations have been reported to be similar in asymptomatic and osteomalacic patients on dialysis. The osteomalacic patients, however, differed in that they failed to elevate their PTH levels in response to a hypocalcemic challenge and exhibited functional hypoparathyroidism.¹⁷⁶ That Al infusions raise total serum Ca levels, yet decrease ionized Ca, is not compatible with hypercalcemia being the mechanism by which Al suppresses PTH production.

Parathyroidectomy can worsen ABD. Sequential bone biopsy specimens in parathyroidectomized dialysis patients have shown accumulation of Al and a decrease in bone formation rates.^{178, 179} In the absence of stainable bone Al, an elevated bone Al content was reported in patients with predominantly hyperparathyroid bone disease.¹⁵³ ABD developed in patients in whom iPTH levels were lowered by treatment with 25(OH)₃ or 1,25(OH)₂D₃.¹²⁷ Also, parathyroidectomized patients with ABD do not respond to DFO therapy as well as those with intact parathyroid glands.¹⁸⁰

Indirect Testing for Aluminum Bone Disease

Following a mean DFO infusion of 28.5 mg/kg to 33 adults, serum Al rose in 18 patients with stainable Al.¹⁵¹ In 15 patients without stainable bone Al, serum Al increased markedly, mildly and not at all in some. Serum Al rose more than 20% in all with stainable Al and less than 20% in those without stainable Al. There was a 33% false positive response to DFO.

In 102 patients, the criteria of a combination of a midrange iPTH value less than 200 $\mu\text{Eq/ml}$ and a post DFO (40/mg/kg) infusion increase in serum Al of 200 $\mu\text{g/L}$ provided a sensitivity of 79% and specificity of 73% for diagnosing ABD.⁵³ Of interest was the lack of correlation between iPTH levels and stainable bone Al. The data demonstrated that plasma Al in-

creases of less than 200 mg/dl and greater than 500 $\mu\text{g/dl}$ correlated well with the absence or presence of ABD, respectively. Intermediate increases in plasma Al, however, require bone biopsies to document the presence or absence of ABD.

DFO Therapy for Aluminum Bone Disease

The effect of DFO therapy on ABD is variable. In patients undergoing hemodialysis, DFO treatment (28 mg/kg IV three times weekly for 10 months) lowered serum Al levels while stainable bone Al increased.¹⁸¹ Histologic parameters of osteomalacia improved, while hyperparathyroidism became worse. An inverse correlation between stainable bone Al and C-terminal PTH was found. The quantity of Al removed by dialysis exceeded that accounted for by changes in bone Al content. Bone scans have been used to follow the progress of DFO therapy.¹⁸²

The impact of DFO on bone histology was evaluated in 28 adults undergoing hemodialysis: improvement occurred in most. The weekly dose of DFO ranged from 2 to 6 gm and duration of therapy ranged from 6 to 18 months; ACPBs were continued throughout therapy to maintain serum phosphate levels of 4.5 to 6.0 $\mu\text{g/dl}$. Marked declines in stainable bone surface area were positively correlated with increases in bone formation rate, bone apposition, and length of double tetracycline labeling.¹⁸⁰ Bone Al content did not decrease and in many cases actually increased, further supporting the concept that the location of Al determines its effect on bone metabolism. Interestingly, the primary type of bone disease changed in 59% of these patients. Patients who had had parathyroidectomies and similar courses of DFO therapy did not respond as well. Even though stainable bone Al decreased, bone formation rates and osteoid volume remained unchanged.

DFO therapy removes bone Al and improves bone histology in children.¹²¹ In a 7.5-year-old child, DFO added to CAPD exchanges (7.5 mg/kg/exchange) for 3 months decreased stainable bone Al from 4.36 to 2.39 mm/sq mm of bone; 85% of the stainable Al that originally was localized to the mineralization front was found in cement lines. Osteoid volume and surface area decreased while mineralization indices improved. In another child, 6 months of chelation therapy (DFO, initially 100 mg/L and then 75 mg/L, added to 8 hourly exchanges of 250 ml for an unspecified time lasting between 13 and 31 months) significantly improved bone histomorphometric indices.¹²³ Comparative studies obtained 6 months apart demonstrated that the osteoid area decreased from 83% to 8%; osteoid surface decreased from 53% to 23%; osteoid seam thickness decreased from 864 to 38 μm , staining converted from positive to negative; and the quantity of calcified bone increased. Serum Al decreased from 1030 to 26 $\mu\text{g/L}$ and bone Al decreased from 494 to 35 mg/kg dry weight. Although DFO improves ABD, criteria for its use are not uniform. Controlled prospective trials without DFO and/or treatment with calcium carbonate (CaCO_3) are not yet available.

Effect of Renal Transplantation on Aluminum Bone Disease

Bone Al content remains elevated after successful renal transplantation. In one study, bone Al content measurements obtained 4 to 60 months after transplantation did not differ significantly from values obtained in a group of patients undergoing chronic hemodialysis.¹³⁰ The bone Al content, however, appeared to be less than expected considering the time patients had been on dialysis.

Anemia

Experimental Studies

The anemia of Al intoxication can be produced experimentally. Uremic and normal rats given intraperitoneal Al for 3 weeks develop microcytosis followed by anemia.¹⁸³ The absence of an accompanying reticulocytosis suggests that Al affects bone erythroid precursors. The pattern of development of anemia with Al is similar to that seen in lead intoxication and iron deficiency.

The precise mechanism leading to decreased heme synthesis remains unclear. Evidence has been presented that δ -aminolevulinic acid dehydratase activity (the second rate-limiting step in heme biosynthesis) is inhibited in vitro but activated in vivo.¹⁸⁴ Lower concentrations of Al may enhance this enzyme while higher concentrations inhibit it.¹⁸⁵ Interaction of Al with zinc may also play a role. In 38 patients on hemodialysis without encephalopathy, erythrocyte dihydropteridine reductase (DHPR) activity was inversely related to serum Al concentrations ($r = -0.61$; $P < 0.001$).¹⁸⁶ After a single dose of DFO, DHPR activity doubled. Another route by which Al may cause anemia is altered iron metabolism. Al is bound to at least one of the specific iron-binding sites of transferrin.¹⁸⁷

Clinical Features

Al exposure in patients with ESRD leads to the development of a microcytic anemia. In adult patients, anemia is more likely when epidemic Al intoxication has occurred. Serum Al levels tend to be higher in anemic patients with advanced renal failure than in those without anemia. Serum Al concentrations averaged 454 $\mu\text{g/L}$ in three reports involving patients with microcytic anemia.¹⁸⁸⁻¹⁹⁰ A decrease in hemoglobin concentration often predates the onset of Al encephalopathy.

In 39 adults in whom serum Al, red blood cell (RBC) Al, serum ferritin, and RBC indices were measured, a significant inverse relationship was found between serum Al and mean corpuscular volume (MCV) as well as between RBC Al and MCV.¹⁹¹ No correlation existed between serum ferritin and RBC Al. After 6.5 months of weekly treatment with 2.0 gm of DFO, mean serum Al decreased from 419 to 209 $\mu\text{g/L}$; MCV increased from 68.7 to 85.7 cu μ , hemoglobin increased from 9.7 to 12.2 gm/dl; and Hct rose from 30.0% to 38.2%. DFO treatment improved MCV both

with and without changes in RBC numbers; the improvement was independent of changes in serum ferritin. These findings support a direct role for Al as a cause of microcytosis. DFO therapy improved microcytosis while serum ferritin levels fell in patients on dialysis.¹⁹² Because parallel falls in plasma Al and RBC Al did not occur, different tissues may release Al at different rates as the Al burden falls.

Data on the relationship between Al and anemia in children are sparse. One study compared six children with serum Al levels exceeding 100 $\mu\text{g/L}$ to 25 children with lower Al levels. The mean hemoglobin level was not significantly lower (7.9 vs. 8.7 gm/dl) in those with higher Al levels.⁴⁴ However, a microcytic anemia was documented in four of six with higher Al levels and only in one of 25 ($p < 0.01$) with lower Al levels. In 12 children with CRF, three of four children with the highest blood Al levels had reduced RBC volumes.⁴⁵

Therapy

DFO therapy in Al-intoxicated children has been associated with improved hemoglobin concentrations. In one child, 3 months of DFO increased MCV to 87 from 63 cu μ and the hemoglobin level to 10.3 from 7.7 gm/dl.¹²¹ In another Al-intoxicated child, DFO increased the hemoglobin level from 7.3 to 12.0 gm/dl and simultaneously normalized a mean corpuscular hemoglobin volume (MCHV) of 62 μm^2 and a mean corpuscular hemoglobin concentration of 20 pg¹²³ with lower Al levels. The mean hemoglobin level was not significantly lower (7.9 vs. 8.7 gm/dl) in those with higher Al levels.⁴⁴ However, a microcytic anemia was documented in four of six with higher Al levels and only in one of 25 ($p < 0.01$) with lower Al levels. In 12 children with CRF, three of four children with the highest blood Al levels had reduced RBC volumes.⁴⁵

Children with CRF may have elevated ferritin and iron loads secondary to repeated transfusions. When DFO therapy for Al intoxication is given, it may simultaneously lower iron stores as measured by decreases in serum ferritin to a level at which supplemental iron therapy is needed. Classical iron deficiency may develop.

Involvement of Other Tissues in ESRD

The incidence of sudden cardiac deaths has been reported to be higher than expected in patients exposed to Al.¹⁹³

An increased incidence of caries has been reported in patients exposed to Al.¹⁹⁴ Children with uremia tend to have a reduced incidence of caries.¹¹⁴

Management of Aluminum Overload in Patients With Renal Failure

Management of patients with Al-related problems involves reductions in exposure to Al, and when indicated, therapy is directed toward increasing

Al removal. Removal of Al by either hemodialysis or peritoneal dialysis clearly improves ABD as well as anemia and encephalopathy in some patients.^{155, 195} These aspects of treatment have already been discussed. This segment focuses on two additional aspects of therapy (1) the effect of DFO on Al kinetics in adults and children undergoing dialysis and (2) ways to reduce exposure to Al.

Aluminum Removal During Dialysis

Al removal in patients undergoing dialysis is enhanced by administering DFO. Desferrioxamine B, a trihydroxamic acid, on contact with heavy metals twines itself around metals to form a hexadentate complex. Al-DFO has a molecular weight of approximately 625 daltons. Compared to ferrioxamine, which has a stability constant (pf) of 10^{31} , Al-DFO has a stability constant (pf) of 10^{22} .¹²² Usually DFO is administered intravenously during hemodialysis. If infused predialyzer, unbound DFO easily traverses the dialysis membrane; when infused postdialyzer, unbound DFO becomes bound as it travels through the blood stream and does not appear in dialysate. Polyacrylonitril hemodialysis membranes allow more Al-DFO to be removed than either cellulose or cupraphane.¹⁹⁶ Al clearances during hemodialysis are significantly enhanced by adding a microencapsulated carbon cartridge in series distal to the dialyzer.¹⁹⁷ It is not yet known whether Al-DFO is fecally excreted.

The quantity of Al removed during a hemodialysis treatment is related to the predialysis serum Al concentration and to DFO given. Al removal during hemodialysis averages 100 $\mu\text{g/hr}$ in the absence of chelation.¹⁹⁸ Further increases without chelation are not possible because only 5% to 20% of Al is ultrafilterable.^{199, 200} The usual dose of DFO in adults is 40 to 80 mg/kg once a week. Lower doses of DFO (15 to 30 mg/kg) given during each dialysis have also been clinically effective.¹¹⁸ After DFO administration, rates of Al removal per hemodialysis can be increased to 20 mg.¹¹⁸

During the dialysis in which DFO is administered, serum Al levels rise considerably, tend to reach maximal levels at 24 hours after infusion, and remain at that level until another dialysis is performed.²⁰¹ Peak Al levels may progressively rise in some patients over many months before starting to fall. In a 10-month-old child, serum Al rose from 151 $\mu\text{g/L}$ to 800 $\mu\text{g/L}$ after 2 months of DFO therapy.⁸¹ The rise in serum Al concentration between dialyses—together with an absence of any Al intake—demonstrates that mobilization of Al from intracellular pools occurs with DFO therapy. In a single center study, intramuscular DFO was shown to be as efficacious as intravenous DFO in removing Al in hemodialysis patients.²⁰² The source of the Al was also shown to derive from tissues other than the RBC.

DFO enhances Al removal during peritoneal dialysis.²⁰³ The amount of Al removed by peritoneal dialysis in patients treated with intravenous or intraperitoneal DFO is similar.²⁰⁴ DFO administration (40 mg/kg) increased

the dialysate to plasma ratio of Al from approximately 0.19 to 0.34. This resulted in a 700% increase in Al removal in the peritoneal effluent. Mean daily Al excretion increased from 218 to 1,521, 1,120, and 946 $\mu\text{g/day}$ during the 3 days, respectively, after giving DFO. The 48-hour accumulative excretion of Al (approximately 2.64 mg) was similar to that achieved during 4 hours of hemodialysis (2.93 mg).¹⁹⁹ In comparing 2.0 gm of DFO given either intravenously or intraperitoneally to adults on CAPD, Al removal was similar (500 to 560 μg per 24 hours).²⁰²

Al removal via dialysate in patients on CAPD reflects the clinical situation. Mean negative Al balance in CAPD patients was 13.5 $\mu\text{g/day}$ in patients not ingesting Al, 54.0 $\mu\text{g/day}$ in those taking ACPB, and 147.3 $\mu\text{g/day}$ after transfer to peritoneal dialysis from hemodialysis.²⁰⁵ In 29 adults on CAPD, Al levels in serum and dialysate measured at 6-month intervals did not change significantly. The patients, however, received ACPBs, throughout the study.⁴⁷ Initial mean serum and dialysate Al levels were 47.3 and 14.4 $\mu\text{g/L}$ (dialysate to plasma ratio of 0.30), respectively. In a single patient on CAPD studied for 3 days after receiving intraperitoneal DFO in the overnight exchange at a dose of 500, 1,000, and 2,000 mg, the 24-hour removal of Al via dialysate was 440, 621, and 883 μg on each day, respectively.²⁰⁶ The effect of DFO on increasing Al removal persisted for at least 14 days. The physiologic explanation for the persistent effect on peritoneal membrane kinetics is not known. Dialysate glucose concentration (convective transport) has a negligible effect on Al mass transport across the peritoneum, averaging 27 and 20 μg per CAPD exchange with 1.5% and 4.25% glucose-containing Dianeal solution, respectively.²⁰⁷

Detailed information on Al removal by various dialysis modalities in children remains limited. In one child, DFO increased Al removal in peritoneal dialysis effluent from 90 to 360 μg per 8 hours and urinary excretion from 50 to 145 μg per 8 hours.¹²³ In a 10-month-old given 40 mg/kg of DFO intraperitoneally once weekly for 3 weeks, Al removal during the 10 hours after DFO increased from 42 to 73 μg , 38 to 70 μg and 30 to 100 μg .⁸¹ The dialysate to plasma Al ratio increased from 0.1 to 0.8. This child had smaller amounts of dialysate drained the day following DFO administration. However, further study is necessary before concluding that DFO alters transmembrane water movement.

DFO also enhances Al removal in patients undergoing hemofiltration.²⁰⁸ In ten children 4 to 15 years old with a mean serum Al level of 32 ± 12 $\mu\text{g/L}$ undergoing hemofiltration, Al balance was negative in the six in whom plasma Al was greater than 25 $\mu\text{g/L}$ and positive in the four in whom plasma Al was less than 15 $\mu\text{g/L}$.²⁰⁹ The Al concentration of the substitution fluid was 4.3 ± 1.1 $\mu\text{g/L}$, and Al removal ranged between 10 and 65 μg per treatment.

The use of DFO is not without risk. Side effects of DFO include anaphylactic reactions, posterior cataracts, abdominal pain, visual loss, and retinal changes similar to retinitis pigmentosa.²¹⁰ Al-intoxicated patients may ex-

perience worsening of bone pain and CNS symptomatology, both of which are dose related. After receiving DFO, some patients developed severe infections, e.g., mucormycosis and infection with *Yersinia enterocolitica*.^{211, 212}

Indications for discontinuing DFO therapy in dialysis patients are imprecise. The decision to discontinue DFO therapy is based on many factors: clinical response, changes in EEG, bone biopsy, serum Al levels, the development of infection, and changes in serum Al levels after a DFO challenge test. Subsequent monitoring is always indicated.

Reductions in Exposure to Aluminum

Monitoring of Al in dialysate and water supplies is necessary to reduce exposure to aluminum. The Association for the Advancement of Medical Instrumentation recommends that the Al concentration in dialysate be kept below 10 $\mu\text{g/L}$. Since reverse osmosis water purification systems reject only 80% to 85% of Al present in tap water, it may also be necessary to use a deionizer to further reduce Al content. Commercial peritoneal dialysate is now available with Al concentrations less than 10 to 15 $\mu\text{g/L}$.²¹³ It may be helpful to monitor the Al concentration of drinking water used by children.

Essential to reducing exposure to ACPBs are reductions in dietary phosphate intake and the use of alternative medications to control serum phosphate concentrations. Attempts should be made to reduce phosphate in the diet to 500 to 1,000 mg/day in children and adolescents. Such diets, however, are quite unpalatable. Low-phosphate-containing formulas are often necessary in infants.

The use of ACPBs should be limited and avoided if possible. CaCO_3 has been shown to reduce absorption of phosphate in patients undergoing dialysis.^{214, 215} In dogs, the addition of 1.5 gm/kg of CaCO_3 added to an oral phosphate load of 50 mg/kg reduces the increment of serum phosphate from 4.0 to 1.9 mg/dl and reduces urinary phosphate excretion by 50%.²¹⁶ In ten uremic children aged 0.5 to 10.5, discontinuation of ACPBs together with the administration of a mean dose of CaCO_3 of 5.7 gm/day (range, 2.5 to 12.8) for 8 to 18 months resulted in a mean serum Ca of 10.2 ± 0.6 mg/dl and serum phosphate of 5.2 ± 0.7 mg/dl.^{217, 218} Plasma Al fell from 96 ± 51 to $36 \pm \mu\text{g/L}$. Results remained similar when the number of children studied was extended to 15.²¹⁹ There were eight instances of hypercalcemia (serum Ca, 11.0 to 11.5 mg/dl). CaCO_3 (mean dose of 97 ± 46 [SD] mg/kg) in conjunction with a dietary intake of phosphorous to 600 mg/dg suppressed hyperparathyroidism in uremic children for up to five years.²²⁰ Vitamin D metabolites were simultaneously given to maintain plasma Ca levels at the upper limit of normal.

Guidelines for using CaCO_3 follow. In a study of 19 children (age range, 10 months to 17 years) ten were on dialysis. CaCO_3 therapy at doses ranging from 600 mg/day to 15 gm/day improved serum phosphate levels, and mean serum Al levels fell from 108.8 to 36.1 $\mu\text{g/L}$ in the ten children

who had been on ACPBs. Hypercalcemia occurred in seven children and responded to either a reduction in vitamin D metabolites or CaCO_3 . Levels of serum alkaline phosphatase and iPTH did not change in those originally taking ACPBs and fell in those treated initially with CaCO_3 .²²¹ The amount of phosphate ingested with each meal should be estimated by a nutritionist and the dose of CaCO_3 titrated accordingly; CaCO_3 should be taken with meals. Ingestion of CaCO_3 can lead to hypercalcemia, diarrhea, constipation, and obstipation. When the Ca times phosphorus product exceeds 70, the patient's serum phosphate levels should be lowered by a combination of a reduction in dietary phosphate and ACPBs if needed. After the calcium phosphate product normalizes, CaCO_3 can be started at a dose of 10 to 20 mg/kg/day and progressively increased to control serum phosphate levels. Serum phosphate levels should be kept in age appropriate normal ranges to avoid hypophosphatemia. Effective doses of CaCO_3 used in children range from 0.1 to 0.3 gm/kg/day in three to four divided doses.²²² If the serum Ca increases to levels above 11.0 mg/dl, ACPBs may be transiently needed or the dose of CaCO_3 or vitamin D metabolite reduced.

Attempts should be made to reduce exposure to other Al-containing drugs, to avoid intravenous additives containing Al, and to avoid infant formulas containing large quantities of Al. Sucralfate, because it contains Al, ought to be avoided in children with ESRD. A low phosphate containing infant formula with a Ca to phosphorous ratio of 1.4 to 2.0:1 should be used.²²³

In summary, it appears that the risk of significant Al intoxication can be reduced or eliminated by substituting CaCO_3 for ACPBs. Insofar as possible, it is recommended that infants and young children not be given ACPBs.

Aluminum in Diseases Other Than Renal

Al is an environmental toxin. Abnormalities of Al metabolism occur in a number of nonrenal disorders.

Aluminum Toxicity and Parenteral Nutrition

Minimum ABD occurs in patients undergoing long-term total parenteral nutrition (TPN) with solutions containing Al-rich additives. Patients with gastrointestinal disease and relatively normal renal function developed bone fractures and pain after receiving TPN for 1 to 3 years during which time their clinical and nutritional status improved significantly.^{224, 225} The source of Al was the casein hydrolysate and the quantity of Al infused was computed to be 2,000 to 3,000 $\mu\text{g/day}$.²²⁶ Determinations of plasma and urinary Al levels led to the conclusion that a significant positive balance of Al had occurred. Laboratory features included hypercalciuria, normal to

slightly increased levels of both serum calcium and phosphorus, iPTH levels that were normal to undetectable, and reduced to undetectable levels of $1,25(\text{OH})_2\text{D}_3$. Bone biopsy specimens revealed Al accumulation at the mineralization fronts, reduced numbers of osteoblasts and osteoclasts, and a reduced bone formation rate as measured by tetracycline labelling. Reductions in the Al content of the TPN solution led to a reduction in bone Al content and improvement in bone formation rate only after 2 to 3 years.²²⁷ These studies clearly establish that Al causes bone disease in the presence of normal renal function. Once large quantities of Al are deposited in bone, its removal takes a long time but demonstrates the reversibility of ABD secondary to Al accumulation.

In 18 premature infants less than 37 weeks of gestation, controls (eight term infants less than 1 month and 35 newborns, and five infants 1.5 to 9 months of age), serum Al levels as well as urinary concentrations of Al and creatinine demonstrated that these infants had been exposed to significant amounts of Al.²²⁸ Plasma Al levels in controls were less than $10 \mu\text{g/L}$; urinary Al levels ranged from 7 to $70 \mu\text{g/L}$ and Al/Cr ratios ranged from 0.1 to 0.28. Al levels in the control umbilical cord samples ($4.5 \pm 3.7 \mu\text{g/L}$) were similar to those obtained during the first month of life. The mean urinary Al/Cr ratio of 0.6 in the normal infants was higher than the expected adult ratio of 0.1. In four infants, urinary Al/Cr ratios remained elevated for 13 days after discontinuing intravenous (IV) therapy. In the five older infants studied after 2 weeks of IV therapy, the estimated Al retention ranged from 60% to 95% of that administered and the urinary Al/creatinine ratio was increased.

Samples for measuring Al were obtained 3 weeks apart in the premature infants. Both plasma Al levels ($36.78 \pm 45.3 \mu\text{g/L}$) and urinary Al/Cr ratios (5.4 ± 4.6) were significantly increased above controls. Two of the premature infants had plasma Al levels greater than $100 \mu\text{g/L}$. Such levels are associated with toxicity. In 13 of the premature infants, mean plasma Al levels fell from 36.2 to $8.1 \mu\text{g/L}$ after they were changed to formula feedings.

In the same study, bone Al content was determined after death in 23 infants; 17 had not received prolonged IV therapy and 6 received at least 3 weeks of parenteral nutrition. Bone Al content in untreated versus treated infants was $1.98 \pm 1.44 \mu\text{g/L}$ and $20.16 \mu\text{g/L} \pm 13.4 \text{ mg/kg}$, respectively. Noteworthy was the single observation that bone Al content was still 300% increased after stopping parenteral therapy for 2 months.

Two groups of ten neonates aged 29 to 41 weeks received solutions containing Al at a mean concentration of either 306 or $144 \mu\text{g/L}$ for 5 to 175 days.²²⁹ Serum Al levels were similar in both groups ($51 \mu\text{g/L}$). Mean urinary Al/Cr ratios increased threefold and were higher in those receiving more Al. Urinary Al/Cr ratios were unrelated to age. It was estimated that only 40% of the infused Al was excreted. In postmortem studies of two infants, their vertebrae stained positive for Al at the mineralization front.

These two studies demonstrate that sick neonates are often exposed to significant amounts of Al. Because infants are not able to excrete the quantities of Al to which they are exposed, they develop an increased body burden of Al as reflected by increased serum Al levels and bone Al content. The increased bone Al content and possibly other tissue stores of Al may persist for a prolonged time. Al exposure in neonates may be reflected by an increased urinary Al/Cr ratio while plasma Al levels are in the normal range. The precise role which Al may play in bone and CNS disease in exposed infants requires additional study.

The quantity of Al in many infant formulas suggests that infants on a per weight basis may ingest five times as much Al as that ingested by infants fed low Al-containing formulas. The urinary Al/Cr ratio data suggest that infants being fed high Al-containing formula may be absorbing relatively more Al than adults. Based on normal infants having plasma and bone Al levels similar to that in adults, it appears that normal infants are able to handle such a load. Conversely, continued exposure to such formulas may lead to Al toxicity. Although other explanations are possible, it is worth noting that soy formulas are associated with an increased incidence of decreased bone mineralization.²³⁰ In a set of 32-week-old twins fed both TPN and Al-containing formula, serum and tissue levels of Al in liver, lung, bone, and brain were considerably increased compared with controls.²³¹ One of the twins had a hypoplastic kidney and a lower GFR. Serum Al levels and urinary Al/Cr ratios were similar in both twins; however, the twin with the hypoplastic kidney had higher tissue levels of Al in three of the four organs examined (less Al in brain).

Al may cause hepatic toxicity. In five children 18 to 34 months of age who received TPN for 18 to 33 months with Al-containing additives, hepatic toxicity developed.²³² Laboratory abnormalities included elevations in total bilirubin, alkaline phosphatase, and serum glutamic oxaloacetic and pyruvic transaminase. Liver biopsy specimens demonstrated bile duct proliferation, brown pigment within hepatocytes, periportal fibrosis, cellular necrosis, and nodular regeneration. Al content in liver tissue was increased 4 to 28 times above control values. Microprobe studies revealed Al in the granules. Rats given an acute Al load of 15/mg/kg/day intraperitoneally develop Al-containing cytoplasmic inclusions.²³³ Similar lesions, however, occur in patients who receive TPN solutions containing much lower quantities of Al. One patient with Al associated dialysis encephalopathy was reported to have inclusions and vacuoles in hepatocytes, periportal fibrosis and mononuclear infiltration.²³³

Aluminum and Primary Neurologic Disorders

Some patients with Alzheimer's disease have been shown to have elevated brain Al levels in both biopsy and postmortem tissue. Brain Al content ranged from 0.40 to 107 mg/kg. Most values are less than those found in patients with ESRD-related encephalopathy. In contrast to renal-related

encephalopathy, patients with Alzheimer's disease have neurofibrillary tangles.^{234, 235} The distribution of Al in Alzheimer's disease is patchy. Higher Al levels are found in regions containing tangles and senile amyloid plaques.²³⁶ Treatment with DFO increases urinary Al excretion and may lower brain Al in patients with Alzheimer's disease. The role of DFO in altering neurologic dysfunction is currently being critically evaluated. Atomic absorption studies and electron probe analysis of brain tissue in patients with Down's syndrome with Alzheimer's disease reveal Al in neurons with tangles.²³⁵ Patients from Guam experience amyotrophic lateral sclerosis and Parkinson-related dementia at rates 30 to 60 times higher than most other areas of the world. An increased Al content was found in these patients in neurons with tangles obtained from the spinal cord.²³⁷

Aluminum and Other Disorders

Brain Al content is elevated in individuals over 70 years of age as well as in patients with metastatic cancer and hepatic coma. Al levels in elderly people are less than 50% of values found in patients with dialysis dementia.²³⁸ Individuals with normal renal function exposed to ACPBs for a long time may accumulate Al in their bones.¹³ Plasma and urinary Al levels are increased in workers exposed to Al.²³⁹ Industrial exposure to Al may be a risk factor for cancer.²⁴⁰ Al together with iron has been found in macrophages in otherwise normal individuals who smoke.²⁴¹

Al exposure may lead to skin telangiectases of the essential type.^{242, 243} In an epidemiologic study of 588 workers in an aluminum plant, 40% had telangiectases on their shoulders, upper chest, and back. A positive correlation between the duration of exposure and the occurrence of lesions was found. The association existed only for those working in the electrolysis division of the plant. It is possible that the skin lesions were secondary to being exposed to one or more additional toxins; however, the report of similar lesions in people working in another Al electrolysis plant in another country is suggestive that the lesions related to Al exposure. Pulmonary lesions, diffuse fibrosis, and foreign body granulomas are described in individuals exposed in various ways to aluminum dust.²⁴⁴ Animal studies confirm that inhalation²⁴⁵ or intratracheal injection²⁴⁶ of Al produces lung disease. The produced lung disease differs somewhat between species.

Al was demonstrated in subcutaneous nodules and granulomas developing after DPT immunization.^{247, 248} Three injections of DPT contain 0.75 to 2.50 mg of Al. The usual three doses of hepatitis B vaccine contains 1.5 mg of Al.²⁴⁹ Federal regulations permit 0.85 to 1.25 mg of Al salts to be used per 0.50 ml of adjuvant and absorbent.

Some lots of plasma concentrates used to treat hemophilia contain significant amounts of Al. Al concentrations exceeding 230 $\mu\text{g/L}$ were found in some batches of factor VIII, factor IX, and intravenous γ -globulin.²⁵⁰ Patients treated with factor concentrates might receive a yearly Al dose exceeding 1 mg/kg while those given IV γ -globulin at a dose of 6 gm/kg

might receive 27 $\mu\text{g/kg}$ of Al. Two patients given factor concentrates had serum Al concentrations of 19 and 10 $\mu\text{g/L}$, respectively (normal values < 4.0 $\mu\text{g/L}$).

Decreased visual-motor performance has been attributed to an increased exposure to Al and Al in combination with lead.²⁵¹ In a study of 69 children in whom the heavy-metal burden was determined by analysis of hair samples, an association between exposure to a number of heavy metals, particularly Al, and an abnormal Bender Visual-Motor Gestalt Test was reported. Others question the validity of using hair samples to ascertain heavy metal burden. In one study, scalp hair was collected from a single individual and cleansed four ways prior to measuring hair Al concentrations. Each way resulted in a different Al concentration.²⁵²

Conclusion

Overwhelming data support the conclusion that the aforementioned clinical findings are due to Al toxicity. The data reviewed relative to Al and renal failure virtually fulfill Koch's postulates for a toxin. Abnormal concentrations of Al are found in affected organs. A reproducible pattern of clinical abnormalities can be correlated with these deposits and reproduced in experimental settings both in vitro and in vivo. Removal of Al results in clinical and histologic improvement and, the reintroduction of Al can worsen clinical symptomatology. Other studies have identified Al as perhaps playing a role in a number of nonrenal disorders. These studies clearly establish Al as another heavy metal intoxicant capable of producing disease in a continuously increasing number of clinical circumstances.

References

1. Alfrey AC, Mishell JM, Burkes J, et al: Syndrome of dyspraxia and multifocal seizures associated with chronic hemodialysis. *Trans Am Soc Artif Intern Organs* 1972; 18:257-261.
2. Alfrey A, LeGendre GR, Kaehny W: The dialysis encephalopathy syndrome: Possible aluminum intoxication. *N Engl J Med* 1976; 294:184-188.
3. Baluarte HJ, Gruskin AB, Hiner L, et al: Encephalopathy in children with chronic renal failure. *Proc Clin Dial Transpl Forum* 1977; 7:95-98.
4. Foley CM, Polinsky MS, Gruskin AB, et al: Encephalopathy in infants and children with chronic renal disease. *Arch Neurol* 1981; 38:656-658.
5. Gruskin AB, Polinsky MS, Lerner GR, et al: Aluminum intoxication in children, in Strauss J (ed): *Renal-GU Disorders: Progression, Replacement Therapy, Growth—Current Concepts in Diagnosis and Management*. Miami, University of Miami Press, 1987, pp 223-232.
6. Finberg L, Dweck HS, Holmes F, et al: Aluminum toxicity in infants and children. Committee on Nutrition. *Pediatrics* 1986; 78(6):1150-1154.
7. Hem JD: Geochemistry and aqueous chemistry of aluminum. *Kidney Int* 1986; 29(18):S3-S7.

8. Savory J, Wills M: Analytical methods for aluminum measurement. *Kidney Int* 1986; 29:S24–S27.
9. Kostyniak PJ: An electrothermal atomic absorption method for aluminum analysis in plasma: Identification of sources of contamination in blood sampling procedures. *J Anal Toxicol* 1983; 7:20–23.
10. Frech W, Cedergren A, Cedergerb C, et al: Evaluation of some critical factors affecting determination of aluminum in blood, plasma, or serum by electrothermal atomic absorption spectroscopy. *Clin Chem* 1982; 28:2259–2263.
11. Berlyne GM, Adler AJ: Serum aluminum cannot be measured accurately. *Am J Kid Dis* 1985; 6(5):288–292.
12. Burnatowska-Hledin M, Mayor GH: Aluminum: Analytical considerations. *Am J Kid Dis* 1985; 6(5):283–287.
13. Gorsky JE, Dietz AA, Spencer H, et al: Metabolic balance of aluminum studies in six men. *Clin Chem* 1979; 25:1739–1743.
14. Recker RR, Blotcky AJ, Leffler JA, et al: Evidence for aluminum absorption from the gastrointestinal tract and bone deposition by aluminum carbonate ingestion with normal renal function. *J Lab Clin Med* 1977; 90:810–815.
15. Ihle BU, Becker GJ: Gastrointestinal absorption of aluminum. *Am J Kid Dis* 1985; 6(5):302–305.
16. Kaehny WD, Hegg AP, Alfrey AC: Gastrointestinal absorption of aluminum from aluminum-containing antacids. *N Engl J Med* 1977; 296:1389–1390.
17. Polinsky MS, Gruskin AB: Aluminum toxicity in children with chronic renal failure. *J Pediatr* 1984; 105:758–761.
18. King SW, Savory J, Wills MR: Aluminum distribution in serum following hemodialysis. *J Clin Lab* 1982; 12:143–149.
19. Gacek EM, Babb AL, Urelli DA, et al: Dialysis dementia: The role of dialysate pH in altering the dializability of aluminum. *Trans Am Soc Artif Intern Organs* 1979; 25:409–411.
20. Henry DA, Goodman WG, Nudelman RK, et al: Parenteral aluminum administration in the dog: I. Plasma kinetics, tissue levels, calcium metabolism, and parathyroid hormone. *Kidney Int* 1984; 25:362–369.
21. Klein GL, Ott SM, Alfrey AC, et al: Aluminum as a factor in the bone disease of long-term parenteral nutrition. *Trans Assoc Am Phys* 1982; 95:155–164.
22. Goodman WG, Henry DA, Horst R, et al: Parenteral aluminum administration in the dog: II. Induction of osteomalacia and effect on vitamin D metabolism. *Kidney Int* 1984; 25:370–375.
23. Rodriguez M, Felsenfeld AJ, Llach F: The role of aluminum in the development of hypercalcemia in the rat. *Kidney Int* 1987; 31:766–771.
24. Henry HL, Norman AW: Interactions between aluminum and the actions and metabolism of vitamin D₃ in the chick. Seventh Annual Scientific Meeting of the American Society for Bone and Mineral Research, Washington, DC, abstract 267, 1985.
25. Trapp GA: Interactions of aluminum with cofactors, enzymes, and other proteins. *Kidney Int* 1986; 29(18):S12–S16.
26. Trapp GA: Studies of aluminum interaction with enzymes and proteins: The inhibition of hexokinase. *Neurotoxicol* 1980; 1:89–100.
27. Hincke MT, Demaille JG: Calmodulin regulation of the ATP-dependent calcium uptake by inverted vesicles prepared from rabbit synaptosomal plasma membranes. *Biochem Biophys Acta* 1984; 771(2):188–194.
28. Suhayda CG, Haug A: Organic acids prevent aluminum-induced conformational

- tional changes in calmodulin. *Biochem Biophys Res Commun* 1984; 119:376–381.
29. Savory J, Wills MR: Dialysis fluids as a source of aluminum accumulation. *Nephron* 1984; 38:12–23.
30. Slanina P, Falkeborn Y, French W, et al: Aluminum concentrations in the brain and bone of rats fed citric acid, aluminium citrate or aluminium hydroxide. *Fd Chem Toxicol* 1984; 22:391–397.
31. Santos F, Malaga S: The risk factors of aluminum intoxication in children. *Hong Kong J Pediatr* 1986; 3:32–37.
32. Robertson JA, Salusky IB, Norris KC, et al: Aluminum absorption in man: Comparison of sucralate and aluminum hydroxide, abstract. *Kidney Int* 1987; 31(1):214.
33. Freundlich M, Zilleruelo G, Abitbol C, et al: Infant formula as a cause of aluminum toxicity in neonatal uraemia. *Lancet* 1985; 2:527–529.
34. Yokel RA: Toxicity of aluminum exposure during lactation to the maternal and suckling rabbit. *Toxicol Applied Pharmacol* 1984; 75:35–43.
35. Santos F, Chan JCM, Yang MS, et al: Aluminum deposition in the central nervous system: Preferential accumulation in the hippocampus in weaning rats. *Med Biol* 1987; 65:53–55.
36. Koning JH: Letter to the editor. *N Engl J Med* 1981; 304:172–173.
37. Poe CF, Leberman JM: The effect of acid foods on aluminum cooking utensils. *Food Technol* 1949; 3:71–74.
38. Trapp GA, Cannon JB: Aluminum pots as a source of dietary aluminum. *N Engl J Med* 1981; 304:172.
39. Adan L, Hainline BW, Jackson DA: The importance of accurate and precise aluminum levels: Letter to the Editor. *N Engl J Med* 1985; 313(25):1609.
40. Jenkins PG: The effect of aluminum antacid ingestion on serum aluminum levels in dialysis patients. *Kidney Int* 1987; 31(1):234.
41. Passlick J, Busch TH, Grabensee B, et al: Elimination of aluminum in patients on CAPD. *Perito Dial Bull* 1986; 6(4):S14.
42. Winney RJ, Cowie JF, Robson JS: Role of plasma aluminum in the detection and prevention of aluminum toxicity. *Kidney Int* 1986; 29(18):S91–S95.
43. Sedman AB, Miller NL, Warady BA, et al: Aluminum loading in children with chronic renal failure. *Kidney Int* 1984; 26:201–204.
44. Trompeter RS, Polinsky MS, Andreoli SA, et al: Neurologic complications of renal failure. *Am J Kid Dis* 1986; 7(4):318–323.
45. Andreoli SP, Bergstein JM, Sherrard DJ: Aluminum intoxication from aluminum-containing phosphate binders in children with azotemia not undergoing dialysis. *N Engl J Med* 1984; 310(17):1079–1084.
46. Mactier RA, Nolph KD, Khanna R, et al: Risk factors for hyperaluminumemia in continuous ambulatory peritoneal dialysis. *Perito Dial Bull* 1986; 6(4):188–193.
47. Gokal R, Ramos JM, Ellis HA, et al: Histological renal osteodystrophy and 25-hydroxycholecalciferol and aluminum levels in patients on continuous ambulatory peritoneal dialysis. *Kidney Int* 1983; 23:15–21.
48. Salusky IB, Coburn JW, Paunier L, et al: Role of aluminum hydroxide in raising serum aluminum levels in children undergoing continuous ambulatory peritoneal dialysis. *J Pediatr* 1984; 105:717–720.
49. Polinsky MS, Kaiser BA, Root AW, et al: The effects of phosphate binder dose on serum aluminum levels in pediatric maintenance dialysis patients. *Pediatr Res* 1986; 20:456A.

50. Chazan JA, Abuelo JG, Blonsky SL: Plasma aluminum levels (unstimulated and stimulated) clinical and biochemical findings in 185 patients on chronic hemodialysis for between 4 and 95 months. *Kidney Int* 1987; 31(1):229.
51. Norris KC, Crooks PW, Nebeker HG, et al: Clinical and laboratory features of aluminum-related bone disease: Differences between sporadic and "epidemic" forms of the syndrome. *Am J Kid Dis* 1985; 6(5):342-347.
52. Coburn JW, Norris KC: Diagnosis of aluminum-related bone disease and treatment of aluminum toxicity with deferoxamine. *Semin Nephrol* 1986; 6(4):S1:12-21.
53. Nebeker HG, Andress DL, Milliner DS, et al: Indirect methods for the diagnosis of aluminum bone disease: Plasma aluminum, the desferrioxamine infusion test, and serum iPTH. *Kidney Int* 1986; 29(18):S96-S99.
54. Crapper DR: Functional consequences of neurofibrillary degeneration, in Terry RD, Gershon S (eds): *Neurobiology of Aging*. New York, Raven Press, 1976, pp 405-432.
55. Petit TL: Aluminum neurobehavioral toxicology, in Dreosti IE, Smith RM (eds): *Neurobiology of the Trace Elements*. Clifton, NJ, Humana Press, 1983, pp 237-274.
56. Petit TL: Aluminum in human dementia. *Am J Kid Dis* 1985; 6(5):313-316.
57. King RG, Chir B, Worland HJ: Desferrioxamine and Alzheimer's dementia. *Med J Aust* 1985; 142(6):352.
58. King GA, DeBoni U, Crapper DR: Effect of aluminum upon conditioned avoidance response acquisition in the absence of neurofibrillary degeneration. *Pharmacol Biochem Behav* 1975; 3:1003-1009.
59. Crapper DR, Quittkat S, Krishnan SS, et al: Intranuclear aluminum content in Alzheimer's disease, dialysis encephalopathy, and experimental aluminum encephalopathy. *Acta Neuropathol (Berl)* 1980; 50:19-24.
60. Wisniewski HM, Sturman JA, Shek JW: Aluminum chloride induced neurofibrillary changes in the developing rabbit: A chronic animal model. *Ann Neurol* 1980; 8:479-490.
61. Petit TL, Biederman GB, Jonas P, et al: Neurobehavioral development following aluminum administration in infant rabbits. *Exp Neurol* 1985; 88(3):640-651.
62. Maher LM, Peterson PL, Chedda RR, et al: The effects of aluminum intoxication in New Zealand white rabbits. Phoenix, AAN Scientific Program Abstract, 1987.
63. Nigro MA, Chhedda R, Lerner G, et al: Encephaloneuromyopathy in chronic aluminum intoxication. *Neurology* 1986; 36:180.
64. Hava M, Hurwitz A: The relaxing effect of aluminum and lanthanum on rat and human gastric smooth muscle in vitro. *Eur J Pharmacol* 1973; 22(2):156-161.
65. Hava M, Hurwitz A: The effect of aluminum chloride on Ca fluxes in isolated longitudinal smooth muscle from rat colon. *Arch Int Pharmacodyn Ther* 1974; 212:24-31.
66. McLachlan DR, Kruck TP, VanBerkum MF: Aluminum and neurodegenerative disease: Therapeutic implications. *Am J Kid Dis* 1985; 6(5):322-329.
67. Farnell BJ, Crapper-McLachlan DR, Baimbridge K, et al: Calcium metabolism in aluminum encephalopathy. *Exp Neurol* 1985; 88:68-83.
68. Alfrey AC: Dialysis encephalopathy. *Clin Nephrol* 1985; 24(1):S15-S19.
69. Arief AL, Fraser CL, Leach W: Cerebral mitochondrial function in uremia: Effects of aluminum, tin, and PTH. *Clin Res* 1984; 32:439A.

70. Burks JS, Alfrey AC, Huddleston J, et al: A fatal encephalopathy in chronic haemodialysis patients. *Lancet* 1976; 1:764-768.
71. Alfrey AC: Dialysis encephalopathy. *Kidney Int* 1986; 29(18):S53-S57.
72. Barron J, Whiteley SJ, Horn AC, et al: A new approach to the early detection of dialysis encephalopathy. *Br J Dis Commun* 1980; 15:75-81.
73. Polinsky MS, Prebis JW, Elzouki AE, et al: The dialysis encephalopathy syndrome in childhood: Results of a survey to determine incidence and geographic distribution of cases. Chronic Renal Disease Conference, National Institute of Arthritis, Metabolism, and Digestive Diseases. Bethesda, MD, January 9-11, 1980.
74. Griswold WR, Reznik V, Mendoza SA, et al: Accumulation of aluminum in a nondialyzed uremic child receiving aluminum hydroxide. *Pediatrics* 1983; 71(1):56-58.
75. Etheridge WB, O'Neill WM Jr: The "dialysis encephalopathy syndrome" without dialysis. *Clin Nephrol* 1978; 10(6):250-252.
76. Mehta RP: Encephalopathy in chronic renal failure appearing before the start of dialysis. *Can Med Assoc J* 1979; 120(9):1112-1114.
77. Smith DB, Lewis JA, Burks JS, et al: Dialysis encephalopathy in peritoneal dialysis. *JAMA* 1980; 244(4):365-366.
78. McKinney TD, Dewberry FL, Stone WJ, et al: Dialysis dementia at the Nashville Veterans Administration Hospital (NVAH). *Abstr Am Soc Nephrol* 1978; 11:46A.
79. Bale JF Jr, Siegler RL, Bray PF: Encephalopathy in young children with moderate chronic renal failure. *Am J Dis Child* 1980; 134:581-583.
80. Geary DF, Fennell RS, Andriola M, et al: Encephalopathy in children with chronic renal failure. *J Ped* 1980; 97(1):41-44.
81. Freundlich M, Zilleruelo G, Faugere M-C, et al: Treatment of aluminum toxicity in infantile uremia with deferoxamine. *J Pediatr* 1986; 109:140-143.
82. Sedman AB, Wilkening GN, Warady BA, et al: Clinical and laboratory observations. *J Pediatr* 1984; 105:836-838.
83. Rotundo A, Nevins TE, Lipton M, et al: Progressive encephalopathy in children with chronic renal insufficiency in infancy. *Kidney Int* 1982; 21:486-491.
84. Polinsky MS, Kaiser BA, Stover JB, et al: Neurologic development of children with severe chronic renal failure from infancy. *Pediatr Nephrol* 1987; 1:157-165.
85. McLaughlin AIG, Kazantzis G, King E, et al: Pulmonary fibrosis and encephalopathy associated with the inhalation of aluminum dust. *Br J Ind Med* 1962; 19:253-263.
86. Parkinson IS, Ward MK, Feest TG, et al: Fracturing dialysis osteodystrophy and dialysis encephalopathy: An epidemiological survey. *Lancet* 1979; 1(8113):406-409.
87. Platts MM, Goode GC, Hislop JS: Composition of the domestic water supply and the incidence of fractures and encephalopathy in patients on home dialysis. *Br Med J* 1977; 2(6088):657-660.
88. Platts MM, Moorhead JH, Greech P: Dialysis dementia. *Lancet* 1973; 2:159.
89. Prior JC, Cameron EC, Knickerbocker WJ, et al: Dialysis encephalopathy and osteomalacic bone disease. *Am J Med* 1982; 72:33-42.
90. Kovalchik MT, Kaehny WD, Jackson T, et al: Aluminum kinetics during hemodialysis. *J Lab Clin Med* 1978; 92(5):712-720.

91. Dunea G, Mahuraker SD, Mamdani B, et al: Role of aluminum in dialysis dementia. *Ann Intern Med* 1978; 88:502.
92. Rozas VV, Port KF, Rutt WM: Progressive dialysis encephalopathy from dialysate aluminum. *Arch Intern Med* 1978; 138(9):1375-1377.
93. Platts MM, Anastassiades E: Dialysis encephalopathy: Precipitating factors and improvement in prognosis. *Clin Nephrol* 1981; 15:223-228.
94. Salusky IB: personal communication, 1987.
95. Sherrard DJ: Aluminum and renal osteodystrophy. *Semin Nephrol* 1986; 6(4):S1:5-11.
96. Alfrey AC: Metabolism and toxicity of aluminum in renal failure. *Am J Clin Nutr* 1980; 33:1509-1516.
97. Elliott HL, Macdougall AI: Dialysis encephalopathy: Evidence implicating aluminum. *Dial Transplant* 1980; 9(11):1027-1030.
98. Williams ED, Boddy K, Harvey I, et al: Calibration and evaluation of a system for total body in vivo activation analysis using 14 MeV neutrons. *Phys Med Biol* 1978; 23:405-415.
99. Alfrey AC: Aluminum metabolism in uremia. *Neurotoxicology* 1980; 1:43-53.
100. Arieff AL, Cooper JD, Armstrong D, et al: Dementia, renal failure, and brain aluminum. *Ann Intern Med* 1979; 90:741-747.
101. Mahoney CA, Arieff AL: Uremic encephalopathies: Clinical, biochemical and experimental features. *Am J Kid Dis* 1982; 2:324-336.
102. McDermott JR, Smith AL, Ward MK, et al: Brain aluminum concentration in dialysis encephalopathy. *Lancet* 1978; 1:901-903.
103. Arieff AI: Aluminum and the pathogenesis of dialysis encephalopathy. *Am J Kid Dis* 1985; 6(5):317-321.
104. Ball JH, Butkus DE, Madison DS: Effect of subtotal parathyroidectomy on dialysis dementia. *Nephron* 1977; 18(3):151-155.
105. Cartier FP, Allain J, Gary J, et al: Progressive myoclonic encephalopathy in dialysis patients. *Nouv Presse Med* 1978; 7(2):97-102.
106. Rivera-Vasquez AB, Noriega-Sanchez A, Ramirez-Gonzalez R, et al: Acute hypercalcaemia in haemodialysis patients: Distinction from "dialysis dementia." *Nephron* 1980; 25:243-246.
107. Nathan E, Pedersen SE: Dialysis encephalopathy in a non-dialysed uraemic boy treated with aluminum hydroxide orally: Case report. *Acta Paediatr Scand* 1980; 69:793-796.
108. Poisson M, Mashaly R, Lebki B: Dialysis encephalopathy, recovery after interruption of aluminium intake. *Br Med J* 1978; 2:1610-1611.
109. McKinney TD, Basinger M, Dawson E, et al: Serum aluminium levels in dialysis dementia. *Nephron* 1982; 32:53-56.
110. Pierides AM, Edwards WG Jr, Cullom UX Jr, et al: Hemodialysis encephalopathy with osteomalacic fractures and muscle weakness. *Kidney Int* 1980; 18:115.
111. O'Hare JA, Callaghan NM, Murnaghan DJ: Dialysis encephalopathy. *Medicine* 1983; 62:129-141.
112. Sideman S, Manor D: The dialysis dementia syndrome and aluminum intoxication. *Nephron* 1982; 31:1-10.
113. Mattern WD, Krisman MR, Blythe WB: Failure of successful renal transplantation to reverse the dialysis-associated encephalopathy syndrome. *Clin Nephrol* 1977; 7(6):275-278.

114. Gruskin AB: Personal observations.
115. Hourmant M, Souillou JP, Boiteau HL, et al: Kinetics of plasma and urine aluminium after renal grafting (author's transl). *Nephrologie* 1981; 2(3):125-129.
116. Boukari M, Jaudon MC, Rottembourg PFG, et al: Kinetics of serum and urinary alum after renal transplantation. *Lancet* 1978; 2:1044.
117. Ackrill P, Ralston AJ, Day JP, et al: Successful removal of aluminum from patients with dialysis encephalopathy. *Lancet* 1980; 2:692-693.
118. Ackrill P, Ralston AJ, Philip-Day J: Role of desferrioxamine in the treatment of dialysis encephalopathy. *Kidney Int* 1986; 29(18):S104-S107.
119. Arze RS, Parkinson IS, Cartledge NEF, et al: Reversal of aluminium dialysis encephalopathy after desferrioxamine treatment. *Lancet* 1981; 2:1116-1119.
120. Milne FJ, Sharfe B, Bell P, et al: The effect of low aluminium water and desferrioxamine on the outcome of dialysis encephalopathy. *Clin Nephrol* 1983; 20:202-207.
121. Andreoli SP, Dunn D, DeMyer W, et al: Intraperitoneal deferoxamine therapy for aluminum intoxication in a child undergoing continuous ambulatory peritoneal dialysis. *J Pediatr* 1985; 107:760-763.
122. Ackrill P, Day JP: Desferrioxamine in the treatment of aluminum overload. *Clin Nephrol* 1985; 24(1):S94-S97.
123. Warady BA, Ford DM, Gaston CE, et al: Aluminum intoxication in a child: Treatment with intraperitoneal desferrioxamine. *Pediatrics* 1986; 78(4):651-655.
124. Kerr DNS, Walls J, Ellis H, et al: Bone disease in patients undergoing regular haemodialysis, abstract. *Br J Bone Joint Surg* 1969; 51B:578.
125. Hodsman AB, Sherrard DJ, Wong EGC, et al: Vitamin D-resistant osteomalacia in hemodialysis patients lacking secondary hyperparathyroidism. *Ann Intern Med* 1981; 94:629-637.
126. Malluche HH, Ritz E, Lange HP, et al: Bone histology in incipient and advanced renal failure. *Kidney Int* 1976; 9:355-360.
127. Coburn JW, Norris KC, Nebeker HG: Osteomalacia and bone disease arising from aluminum. *Semin Nephrol* 1986; 6(1):68-89.
128. Chan Y, Alfrey AC, Posen A, et al: The effect of aluminum on normal and uremic rats: Tissue distribution, vitamin D metabolites and quantitative bone histology. *Am J Kid Dis* 1983; 23:344-351.
129. Robertson JA, Felsenfeld AJ, Haygood CC, et al: Animal model of aluminum-induced osteomalacia: Role of chronic renal failure. *Kidney Int* 1983; 23:327-335.
130. Ellis HA, McCarthy JH, Herrington J: Bone aluminium in haemodialysed patients and in rats injected with aluminium chloride: Relationship to impaired bone mineralisation. *J Clin Pathol* 1979; 32:832-844.
131. Berlyne GM, Ben-Ari J, Knopf E, et al: Aluminum toxicity in rats. *Lancet* 1972; 1(750):564-568.
132. Goodman WG, Gilligan JM: Thyroparathyroidectomy modifies the skeletal response to aluminum loading in the rat. *Kidney Int* 1987; 31:923-929.
133. Quarles LD, Dennis VW, Gitelman HJ, et al: Aluminum deposition at the osteoid-bone interface: An epiphenomenon of the osteomalacia state in vitamin D-deficient dogs. *J Clin Invest* 1985; 75:1441-1447.
134. Plachot JJ, Cournot-Witmer G, Halpern A, et al: Bone ultrastructure and x-

- ray microanalysis of aluminum-intoxicated hemodialysis patients. *Kidney Int* 1984; 25:796–803.
135. Clarkson EM, Lusk VA, Hynson WV, et al: The effect of aluminum hydroxide on calcium, phosphorus and aluminum balances, the serum parathyroid hormone concentration and the aluminum content of bone in patients with chronic renal failure. *Clin Sci* 1972; 43:519–531.
136. Thomas WC, Meyer JL: Aluminum-induced osteomalacia: An explanation. *Am J Nephrol* 1984; 4:201–203.
137. Netter P, Kessler M, Burnel D, et al: Aluminum in the joint tissues of chronic renal failure patients treated with regular hemodialysis and aluminum compounds. *J Rheumatol* 1984; 11:66–70.
138. Thomas WC Jr: Trace metal-citric acid complexes as inhibitors of calcification and crystal formation. *Proc Soc Exp Biol Med* 1982; 170:321–327.
139. Miyahara T, Hayashi M, Kozuka H: The effect of aluminum on the metabolism of embryonic chick bone in tissue culture. *Toxicol Letters* 1984; 21:237–240.
140. Posner AS, Blumenthal NC, Boskey AL: Model of aluminum-induced osteomalacia: Inhibition of apatite formation and growth. *Kidney Int* 1986; 18:S17–S19.
141. Posner AS, Matyas JR, Blumenthal NC: In vitro model of aluminum induced osteomalacia: Inhibition of apatite formation and growth. 30th Annual Meeting of Orthopedic Research Society, 1984, vol 8, no 2, pp 273–274.
142. Lieberherr M, Grosse B, Cournot-Witmer G, et al: In vitro effects of aluminum on bone phosphatases: A possible interaction with PTH and vitamin D₃ metabolites. *Calcif Tissue Int* 1982; 34:202–216.
143. Lieberherr M, Grosse B, Cournot-Witmer G, et al: Aluminum action on mouse bone cell metabolism and response to PTH and 1,25(OH)₂D₃. *Kidney Int* 1987; 31:736–743.
144. Merke J, Lucas PA, Szabo A, et al: 1,25(OH)₂D₃ receptors and endorgan response in experimental aluminium intoxication. *Kidney Int* 1987; 32:204–211.
145. Ellis HA: Aluminum and osteomalacia after parathyroidectomy. *Ann Intern Med* 1982; 96:533–534.
146. Chesney RW, Mehls O, Anast CS, et al: Renal osteodystrophy in children: The role of vitamin D, phosphorus, and parathyroid hormone. *Am J Kid Dis* 1986; 7(4):275–284.
147. Maloney NA, Ott SM, Alfrey AC, et al: Histological quantitation of aluminum in iliac bone from patients with renal failure. *J Lab Clin Med* 1982; 99(2):206–216.
148. Visser WJ, Van de Vyver FL: Aluminium-induced osteomalacia in severe chronic renal failure (SCRF). *Clin Nephrol* 1985; 24(1):S30–S36.
149. Irwin DA: The demonstration of aluminum in animal tissues. *Arch Indust Health* 1955; 12:218–220.
150. Faugere M-C, Malluche HH: Stainable aluminum and not aluminum content reflects bone histology in dialyzed patients. *Kidney Int* 1986; 30:717–722.
151. Malluche HH, Faugere M-C: Aluminum: Toxin or innocent bystander in renal osteodystrophy. *Am J Kid Dis* 1985; 6(5):336–341.
152. Pierce-Myli M, Pierides A: Iron and aluminum osteomalacia during hemodialysis. *Kidney Int* 1984; 25:151.
153. Cournot-Witmer G, Zingraff J, Plachot JJ, et al: Aluminum localization in

- bone from hemodialyzed patients: Relationship to matrix mineralization. *Kidney Int* 1981; 20:375–385.
154. Ott SM, Maloney NA, Coburn JW, et al. The prevalence of bone aluminum deposition in renal osteodystrophy and its relation to the response to calcitriol therapy. *N Engl J Med* 1982; 307:709–713.
155. Malluche HH, Smith AJ, Abreo K, et al. The use of deferoxamine in the management of aluminum accumulation in bone in patients with renal failure. *N Engl J Med* 1984; 311:140–144.
156. Milliner DS, Nebeker HG, Ott SM, et al. Use of deferoxamine infusion test in the diagnosis of aluminum-related osteodystrophy. *Ann Intern Med* 1984; 101:755–780.
157. Llach F, Felsenfield AJ, Coleman MD, et al. The natural course of dialysis osteomalacia. *Kidney Int* 1986; 29(18):S74–S79.
158. Sherrard DJ, Ott SM, Adress DL. Pseudohyperparathyroidism: Syndrome associated with aluminum accumulation in patients with renal failure. *Am J Med* 1985; 79:127–130.
159. Boyce BF, Fell GS, Elder HY, et al. Hypercalcemic osteomalacia due to aluminum toxicity. *Lancet* 1982; 2:1009–1013.
160. Eastwood JB, Bordier PJ, DeWardener HE. Some biochemical, histological, radiological and clinical features of renal osteodystrophy. *Kidney Int* 1973; 4:128–140.
161. Haussler MR, McCain TA. Basic and clinical concepts related to vitamin D metabolism and action. *N Engl J Med* 1977; 297:974–983, 1041–1050.
162. Shimida H, Nakamura M, Morumo F. Influence of aluminum on the effect of 1- α (OH) D_3 on renal osteodystrophy. *Nephron* 1983; 35:163–170.
163. Hodsman AB, Hood SA, Brown P, et al. Do serum aluminum levels reflect underlying skeletal aluminum accumulation and bone histology before or after chelation by deferoxamine? *J Lab Clin Med* 1985; 106(6):674–681.
164. Wright RS, Mehls O, Ritz E, et al. Musculoskeletal manifestations of chronic renal failure, dialysis, and transplantation, in Bacon P, Nadler N (eds): *Renal Manifestations in Rheumatic Disease*. London, Butterworth, 1982, pp 352–384.
165. Olgaard K, Heerfordt J, Madsen S. Scintigraphic skeletal changes in uremic patients on regular hemodialysis. *Nephron* 1976; 17:325–334.
166. Andreoli SP, Smith JA, Bergstein JM. Aluminum bone disease in children: Radiographic features from diagnosis to resolution. *Radiology* 1985; 156:663–667.
167. Salusky IB, Goodman WG, Brill J, et al. Modification of osteitis fibrosa (OF) by aluminum (Al): Studies in children on CAPD. *Pediatr Res* 1986; 20:456A.
168. Morrissey J, Rothstein M, Mayor G, et al. Suppression of parathyroid hormone secretion by aluminum. *Kidney Int* 1983; 23:699–704.
169. Cann CE, Prussin SG, Gordan GS. Aluminum uptake by the parathyroid glands. *J Clin Endocrinol Metab* 1979; 49:543–545.
170. Kanis JA, Earnshaw M, Heynen G, et al. Changes in histological and biochemical indexes of bone turnover after bilateral nephrectomy in patients on hemodialysis: Evidence for a possible role of endogenous calcitonin. *N Engl J Med* 1977; 296:1073–1079.
171. Bourdeau A, Plachot JJ, Courmot-Witmer G, et al. In vitro effects of aluminum on parathyroid glands: Correspondence between hormonal secretion and ultrastructural aspects, abstract. Eighth International Conference on Ca Regulating Hormones. Kobe, Japan 1983.

172. Bellorin-Font E, Weaver ME, Stokes TJ, Jr, et al: Effects of aluminum on bovine parathyroid adenylate cyclase. *Endocrinology* 1985; 117:1456–1461.
173. Bourdeau AM, Bourdon R, Kindermans C, et al: Effects of aluminum addition on parathyroid tissue incubation medium composition. *Kidney Int* 1986; 29:924–926.
174. Mendes V, Jorsetti V, Nemeth J, et al: Secondary hyperparathyroidism in chronic haemodialysis patients: A clinico-pathological study. *Proc Eur Dial Transplant Assoc* 1983; 20:731–738.
175. Bourdeau AM, Plachot J-J, Cournot-Witmer G, et al: Parathyroid response to aluminum in vitro: Ultrastructural changes and PTH release. *Kidney Int* 1987; 31:15–24.
176. Kraut JA, Shinaberger JH, Singer FR, et al: Parathyroid gland responsiveness to acute hypocalcemia in dialysis osteomalacia. *Kidney Int* 1983; 23:725–730.
177. Andress D, Felsenfeld AJ, Voists A, et al: Parathyroid hormone response to hypocalcemia in hemodialysis patients with osteomalacia. *Kidney Int* 1983; 24(3):364–370.
178. Andress DL, Ott SM, Maloney NA, et al: Effect of parathyroidectomy on bone aluminum accumulation in chronic renal failure. *N Engl J Med* 1985; 312:468–473.
179. De Vernejoul MC, Marchais S, London G, et al: Increased bone aluminum deposition after subtotal parathyroidectomy in dialyzed patients. *Kidney Int* 1985; 27:785–791.
180. Andress DL, Nebeker NG, Ott SM, et al: Bone histologic response to deferoxamine in aluminum-related bone disease. *Kidney Int* 1987; 31:1344–1350.
181. Smith AJ, Faugere MC, Fanti P, et al: Trade in and trade off of deferoxamine (DFO) therapy in hemodialyzed patients. *Kidney Int* 1987; 31(1):246.
182. Iwamoto N, Ono T, Yamazaki S, et al: Clinical features of aluminum-associated bone disease in long-term hemodialysis patients. *Nephron* 1986; 42:204–209.
183. Kaiser L, Schwartz KA, Burnatowska-Hledin MA, et al: Microcytic anemia secondary to intraperitoneal aluminum in normal and uremic rats. *Kidney Int* 1984; 26:269–274.
184. Garnica AD: Trace metals and hemoglobin metabolism. *Ann Clin Lab Sci* 1981; 11:220–228.
185. Meredith PA, Moore MR, Goldbert A: The effects of aluminum, lead and zinc on delta-aminolaevulinic acid dehydratase. *Biochem Soc Trans* 1974; 2:1243–1245.
186. Altmann P, Al-Salihi F, et al: Serum aluminum levels and erythrocyte dihydropteridine reductase activity in patients on hemodialysis. *N Engl J Med* 1987; 317:80–84.
187. Trapp GA: Plasma aluminum is bound to transferrin. *Life Sci* 1983; 33:3111–3116.
188. Elliot HL, McDougall AI: Aluminum studies in dialysis encephalopathy. *Proc Eur Dial Transplant Assoc* 1978; 15:157–163.
189. Short A, Winney RJ, Robson JS: Reversible microcytic hypochromic anaemia in dialysis patients due to aluminum intoxication. *Proc Eur Dial Transplant Assoc* 1980; 17:226–233.
190. O'Hare JA, Murnaghan DJ: Reversal of aluminum induced hemodialysis anemia by low-aluminum dialyzate. *N Engl J Med* 1982; 306:654–656.

191. Abreo K, Trapp GA, Wilson R, et al: Diagnosis and therapy of aluminum (AL) induced microcytic anemia (MA) in patients undergoing hemodialysis (HD), abstract. *Kidney Int* 1987; 31(1):225.
192. Swartz R, Dombrowski J, Burnatowska-Hledin M, et al: Microcytic anemia in dialysis patients: Reversible marker of aluminum toxicity. *Kidney Int* 1987; 9(3):217-223.
193. Elliott HL, Macdougall AI, Fell GS: Aluminium toxicity syndrome. *Lancet* 1978; 1:1203.
194. Vrbic V, Stupar J: Dental caries and the concentration of aluminum and strontium in enamel. *Caries Res* 1980; 14(3):141-147.
195. Bertholf RL, Roman JM, Brown S, et al: Aluminum hydroxide-induced osteomalacia, encephalopathy and hyperaluminemia in CAPD: Treatment with desferrioxamine. *Perit Dial Bull* 1984; 1:30.
196. Chang TMS, Barre P: Effect of desferrioxamine on removal of aluminum and iron by coated charcoal haemoperfusion and haemodialysis. *Lancet* 1983; 2:1051-1053.
197. Slatopolsky E, Weerts C, Finch J, et al: The use of microencapsulated carbon in the removal of aluminum in dialysis patients. (abstract). *Kidney Int* 1986; 29:226.
198. Hodge KC, Day JP, O'Hara M, et al: Critical concentration of aluminium in water used for dialysis. *Lancet* 1981; 2:802-803.
199. Milliner DS, Hercz G, Miller JH, et al: Clearance of aluminum by hemodialysis: Effect of desferrioxamine. *Kidney Int* 1986; 29:S100-S103.
200. Graf H, Stummvoll HK, Meisner V, et al: Aluminum removal by hemodialysis. *Kidney Int* 1981; 19(4):587-592.
201. Milliner DS, Alfrey AC, Sherrard DJ, et al: Responses to desferrioxamine infusion in patients with dialysis osteomalacia, in Frame B, Potts JT (eds): *Clinical Disorders of Bone and Mineral Metabolism*. Amsterdam, Excerpta Medica, 1983, p 487.
202. Molitoris BA, Alfrey PS, Miller NL, et al: Efficacy of intramuscular and intraperitoneal deferoxamine for aluminum chelation. *Kidney Int* 1987; 31:986-991.
203. Williams P, Khanna R, Crapper-McLachlan DR: Enhancement of aluminum removal in a patient on CAPD with dementia. *Perito Dial Bull* 1981; 1:73.
204. Hercz G, Salusky IB, Norris KC, et al: Aluminum removal by peritoneal dialysis: Intravenous vs. intraperitoneal deferoxamine. *Kidney Int* 1986; 30(6):944-948.
205. Rottembourg J, Gallego JL, Jaudon M, et al: Serum concentration and peritoneal transfer of aluminum during treatment by continuous ambulatory peritoneal dialysis. *Kidney Int* 1984; 25:919-924.
206. Taber T, Hegeman T, York S, et al: Removal of aluminum with intraperitoneal deferoxamine. *Perito Dial Bull* 1986; 6(4):213.
207. Sorkin MI, Nolph KD, Anderson HO, et al: Aluminium mass transfer during CAPD. *Perito Dial Bull* 1981; 1:91-93.
208. Mason JC, Jones NC, Hilton PJ: Aluminum in haemofiltration solutions. *Lancet* 1983; 1:762-763.
209. Bettinelli A, Buratti M, Elicio T, et al: Aluminum and zinc exchange in children on chronic hemofiltration. *Blood Purif* 1984; 2:45-46.
210. Davies SC, Hungerford JL, Arden GB, et al: Ocular toxicity of high dose intravenous desferrioxamine. *Lancet* 1983; 2:181-184.

211. Boyce N, Wood C, Holdsworth S, et al: Life threatening sepsis complicating heavy metal chelation therapy with deferoxamine. *Aust NZ J Med* 1985; 15:654-655.
212. Eiser AR, Slifkin RF, Neff MS: Intestinal mucormycosis in hemodialysis patients following deferoxamine. *Am J Kid Dis* 1987; 10(1):71-73.
213. Mion C: Aluminium in continuous ambulatory peritoneal dialysis and post dilutional hemofiltration. *Clin Nephrol* 1985; 24(1):S88-S93.
214. Slatopolsky E, Weerts C, Lopez-Hilker S, et al: Calcium carbonate as a phosphate binder in patients with chronic renal failure undergoing dialysis. *N Engl J Med* 1986; 315:157-161.
215. Addison JF, Foulks CJ: Calcium carbonate: An effective phosphorus binder in patients with chronic renal failure. *Curr Ther Res* 1985; 38:241-249.
216. Lopez S, Galceran T, Slatopolsky E: Evaluation of calcium carbonate as an effective phosphorus-binder in the dog. *Clin Res* 1984; 32:452A.
217. Salusky IB, Coburn JW, Foley J, et al: Calcium carbonate as a phosphate-binder in children on dialysis. *Kidney Int* 1985; 27:185.
218. Slatopolsky E: The interaction of parathyroid hormone and aluminum in renal osteodystrophy. Nephrology Forum. *Kidney Int* 1987; 31:842-854.
219. Salusky IB, Coburn JW, Foley J, et al: Effects of oral calcium carbonate on control of serum phosphorus and changes in plasma aluminum levels after discontinuation of aluminum-containing gels in children receiving dialysis. *J Pediatr* 1986; 108:767.
220. Tamanaha K, Mak RHK, Rigden SPA, et al: Long-term suppression of hyperparathyroidism by phosphate binders in uremic children. *Pediatr Nephrol* 1987; 1:145-149.
221. Andreoli SP, Dunson JW, Bergstein JM: Calcium carbonate is an effective phosphorus binder in children with chronic renal failure. *Am J Kid Dis* 1987; 9(3):206-210.
222. Alon U, Davidai G, Bentur L, et al: Oral calcium carbonate as phosphate-binder in infants and children with chronic renal failure. *Miner Electrolyte Metab* 1986; 12:320-325.
223. Santos F, Massie MD, Chan JCM: Risk factors in aluminum toxicity in children with chronic renal failure. *Nephron* 1986; 42:189-195.
224. Klein GL, Ament ME, Bluestone R, et al: Bone disease associated with total parenteral nutrition. *Lancet* 1980; 2:1041-1044.
225. Shike M, Harrison JG, Sturtridge WT, et al: Metabolic bone disease in patients receiving long-term parenteral nutrition. *Ann Intern Med* 1980; 92:343-350.
226. Klein GL, Alfrey AC, Miller NL, et al: Aluminum loading during total parenteral nutrition. *Am J Clin Nutr* 1982; 35:1425-1429.
227. Ott SM, Maloney NA, Klein GL, et al: Aluminum is associated with low bone formation in patients on chronic parenteral nutrition. *Ann Intern Med* 1983; 98:910-914.
228. Sedman AB, Klein GL, Merritt RJ, et al: Evidence of aluminum loading in infants receiving intravenous therapy. *N Engl J Med* 1985; 312:1337-1343.
229. Koo W, Kaplan L, Bendon R, et al: Response to aluminum in parenteral nutrition during infancy. *Pediatr Res* 1986; 20:352A.
230. Kohler L, Meeuwisse G, Mortensson W: Food intake and growth of infants between six and twenty-six weeks of age on breast milk, cow's milk formula or soy formula. *Acta Paediatr Scand* 1984; 73:40-48.

231. Bozynski MEA, Sedman AB, Naglie RA, et al: Serial serum and urinary aluminum levels and tissue loading in preterm twins. *Pediatr Res* 1987; 21:424A.
232. Klein GL, Berquist WE, Ament ME, et al: Hepatic aluminum accumulation in children on total parenteral nutrition. *J Pediatr Gastroenterol Nutr* 1984; 3:740-743.
233. Galle P, Giudicelli CP: Toxicite de l'aluminium pour l'hepatocyte: Localisation ultrastructurale et microanalyse des depots. *Nouv Presse* 1982; 11:1123-1125.
234. Crapper DR, Krishnan SS, Dalton AJ: Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. *Science* 1973; 180:511-513.
235. Crapper DR, Krishnan SS, Quittkat S: Aluminum neurofibrillary degeneration and Alzheimer's disease. *Brain* 1976; 99:67-80.
236. Crapper-McLachlan DR, Kruck TPA, VanBerkum MFA: Aluminum and neurodegenerative disease: Therapeutic implications. *Am J Kid Dis* 1985; 6(5):322-329.
237. Yoshimasu F, Nebayashi Y, Yase Y, et al: Studies on amyotrophic lateral sclerosis by neutron activation and analysis. *Folia Psych et Neurol Jap* 1976; 30:49-55.
238. Arief AI: Neurological manifestations of uremia, in Brenner BM, Rector FC, Jr (eds): *The Kidney*. Philadelphia, WB Saunders, 1986, pp 1731-1756.
239. Sjogren B, Lundberg I, Lidums V: Aluminium in the blood and urine of industrially exposed workers. *Br J Ind Med* 1983; 40(3):301-304.
240. Anderson A, Dahlberg BE, Magnus K, et al: Risk of cancer in the Norwegian aluminium industry. *Int J Cancer* 1982; 29(3):295-298.
241. Brody AR, Craighead JE: Cytoplasmic inclusions in pulmonary macrophages of cigarette smokers. *Lab Invest* 1975; 32(2):125-132.
242. Theriault G, Cordier S, Harvey R: Skin telangiectases in workers at an aluminum plant. *N Engl J Med* 1980; 303:1278-1281.
243. McGrae JD, Jr, Winkelmann RK: Generalized essential telangiectasia: Report of a clinical and histochemical study of 13 patients with acquired cutaneous lesions. *JAMA* 1963; 185:909-913.
244. Van de Vyver FL, De Broe ME: Aluminum in tissues. *Clin Nephrol* 1985; 24(1):S37-S57.
245. Gross P, Harley RA, de Treville RTP: Pulmonary reaction to metallic aluminum powders: An experimental study. *Arch Environ Health* 1973; 26(5):227-236.
246. King EJ, Harrison CV, Mohanty GP, et al: The effect of various forms of alumina on the lungs of rats. *J Path Bact* 1955; 69:81-93.
247. Erdohazi M, Newman RL: Aluminum hydroxide granuloma. *Br Med J* 1971; 3(775):621-623.
248. Slater DN, Underwood JCE, Durrant TE, et al: Aluminum hydroxide granulomas: Light and electron microscopic studies and x-ray microanalysis. *Br J Dermatol* 1982; 107(1):103-108.
249. Lione A: More on aluminum in infants: Letter to the Editor. *N Engl J Med* 1986; 314:923.
250. Bussel J, Alcock N: Aluminum contamination of plasma concentrates. *Pediatr Res* 1987; 21:297A.
251. Marlowe M, Steller J, Errera J, et al: Main and interaction effects of metal

- pollutants on visual-motor performance. *Arch Environ Health* 1985; 40:221–225.
252. DeGroot HJ, DeHaas EJM, D'Haese P, et al: Determination by flameless atomic absorption of aluminium in serum and hair for toxicology monitoring of patients on chronic intermittent hemodialysis. *Pharm Weekbl Sci Ed* 1984; 6:11.
253. Gilli P, Farinelli A, Fagioli F, et al: Serum aluminum levels in patients on peritoneal dialysis. *Lancet* 1980; 2:742–743.
254. Thomson NM, Stevens BJ, Humphrey TJ, et al: Comparison of trace elements in peritoneal dialysis, haemodialysis and uraemia. *Kidney Int* 1983; 23:9–14.
255. Stummvoll HK, Graf H: Aluminum kinetics during renal replacement therapies. *Am J Kid Dis* 1985; 6:293–296.

Behavioral Alterations in Iron Deficiency*

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Evidence that iron deficiency has important behavioral effects has steadily accumulated in the past decade. The resulting picture of behavioral alterations due to iron deficiency reflects the convergence of two independent but complementary investigational approaches: studies of central nervous system biochemical changes, primarily in the laboratory animal, and studies of behavior before and after iron treatment, primarily in the young human. The several studies have yielded a set of findings that has been reproducible in broad general outline. However, many specific results still await replication, and the research is largely at the stage of generating, rather than testing, hypotheses about underlying mechanisms. This is a dynamic area, and substantial clarification may be forthcoming in the next decade. To underscore both the findings about which there is a growing consensus and the issues requiring further study, biochemical work related to behavior is summarized and recent studies directly assessing human behavior are then considered, describing in most detail those involving children.

Biochemical Bases for Altered Behavior

Iron is present in tissue both in compounds known to have metabolic or enzymatic function and in compounds related to iron transport and storage.^{1,2} Examples of metabolically active compounds include the heme proteins, all of which have a role in oxidative metabolism (hemoglobin, myoglobin, mitochondrial cytochromes, and heme-containing enzymes), and some nonheme iron compounds, such as flavoproteins and sulfur-containing proteins. The primary compounds involved in storing iron are ferritin and hemosiderin, and transferrin is the major vehicle for transporting iron. In addition to these iron-containing compounds are compounds that do not contain iron but require iron or heme as cofactors. That this

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latter group of compounds contains enzymes important in the metabolism of the biogenic amines has led to a number of biochemical studies of the effects of iron deficiency.

Iron Deficiency and Biogenic Amines in the Central Nervous System

Nonheme iron is unevenly distributed in the human brain. It is highly localized in extrapyramidal regions (globus pallidus, substantia nigra, putamen, red nucleus, thalamus, and caudate nucleus), with concentrations in certain areas exceeding that in the liver.³ Much of the biochemical work has been in the rat; distribution of brain iron in the rat is similar to that in the human.⁴ Most of the available iron in the brain seems to be in the ferric form. Light-microscopic studies of the globus pallidus and substantia nigra indicate that iron is deposited in fine granular structures in neutrophils, oligodendrocytes, and nerve cells.⁵ In the rat brain, synaptosomal and myelin fractions contain the highest levels of iron.⁶

Brain iron accumulates from birth to early adulthood.³ Perhaps for this reason, brain iron levels are more seriously affected by iron deficiency in the very young animal than in the adult. For example, it has been found that a brief period of severe iron deficiency in the young rat, but not in the adult, resulted in a deficit of brain iron that was not corrected by iron therapy, even though all signs of systemic iron deficiency were reversed.⁷⁻¹¹ The failure to reverse iron depletion in the brain with iron treatment seems to be due to a slow rate of replacement of brain iron compounds.¹²

Recent research on the effects of iron deficiency on central biogenic amines has identified iron-related alterations with increasing specificity. Iron deficiency alters neurotransmission involving dopamine.⁵ The large corpus of research on dopamine and its behavioral effects thus becomes directly relevant to research on iron deficiency.¹³⁻¹⁵

Dopamine is the major neurotransmitter of the extrapyramidal system of the brain. Pharmacologic agents are known to alter dopaminergic transmission in a variety of ways. These agents may prevent amine storage in the synaptic vesicles, provide precursors to replenish amine stores, inhibit amine synthesis by blocking enzymes, have specific amine neurotoxic effects, antagonistically block receptors, or agonistically activate receptors. These pharmacologically induced changes in dopaminergic activity also have behavioral concomitants. The results of numerous studies of behavioral changes when the dopamine system is altered "suggest that forebrain pathways containing the neurotransmitter dopamine are crucial elements in the neural substrate which allows an animal to emit appropriate responses to meaningful stimuli."¹³ Spontaneous motor behavior of the rat in a novel environment is dependent on the dopaminergic system, and forebrain dopamine pathways are essential for the initiation of normal sequences of unconditioned behavior, such as spontaneous locomotor activ-

ity, feeding, and drinking. Learned motor behaviors are also affected, and some aspects of cognitive function may be impaired.^{13, 16}

Research linking iron deficiency and dopaminergic function has come primarily from animal experiments in the laboratory of Youdim and associates.⁵ This research was stimulated by studies that implicated iron as a cofactor for tryptophan hydroxylase and tyrosine hydroxylase, the rate-limiting enzymes in the biosynthesis of serotonin and the catecholamines (dopamine, epinephrine, and norepinephrine), respectively,^{1, 2} and by observations of lowered activity of monoamine oxidase, including changes in platelets of iron deficient subjects.¹⁷ Although initial expectations were that monoamine neurotransmitter levels would be altered due to iron's role in these enzymes, such changes have not been consistently observed in the brain. Mackler et al.¹⁸ did note some changes in aldehyde oxidase and serotonin levels, but Youdim and associates^{10, 19} found no effects of iron deficiency on the activities of such key enzymes in the central nervous system as monoamine oxidase, tyrosine or tryptophan hydroxylase, succinate dehydrogenase, and aldehyde oxidase or on either the levels or turnover rates of norepinephrine, dopamine, and serotonin, even when brain nonheme iron content was lowered by 40%.

Despite finding these biochemical parameters unchanged in iron-deficient rats, Youdim and associates^{10, 19} observed differences in behavior similar to those found when serotonin or dopamine levels are altered. Increased motor activity and stereotypic movements (head movements, circling, and biting) are well-characterized behavioral syndromes induced by pharmacologically increasing brain synthesis of serotonin and dopamine, by using serotonin or dopamine agonists, or by administering a dopamine-releasing drug. Iron-deficient rats showed significant reductions in these syndromes, and their behavior rapidly returned to normal after iron supplementation.^{10, 19} The iron-deficient animal seemed to resemble one treated with drugs that diminish dopaminergic activity—dopamine receptor-blocking agents or neuroleptics, such as haloperidol and fluphenazine. Specifically, iron-deficient rats, like those treated with neuroleptics, showed reduced motor activity after receiving the centrally acting drugs apomorphine and d-amphetamine,^{10, 19–21} inhibition of d-amphetamine-induced hypothermia,^{10, 21} an increase in sleeping time when given barbiturates,²¹ and increases in both the prolactin-binding sites in the liver and the prolactin level in the serum.^{22, 23} The changes in prolactin provide evidence that diminished dopaminergic activity is observed in the pituitary, as well as in the extrapyramidal system.⁵

Taking advantage of the variety of drugs that are known to increase or block dopamine activity, Youdim and colleagues attempted, in a connected series of experiments, to narrow the possible points at which iron deficiency has its effect. It seems that iron deficiency alters the function of a specific postsynaptic dopamine receptor.^{5, 10, 19–21, 24} Because the levels of the neurotransmitters and the enzymes involved in their metabolism were basically unaffected by iron deficiency, it was suggested that altered

postsynaptic response to the released neurotransmitters provided the explanation for the alteration in behavioral responses after pharmacologic manipulations. The two types of postsynaptic dopamine receptors, D_1 and D_2 , were then studied. Dopamine-sensitive adenylate cyclase activity in the rat caudate nucleus, part of the presumed D_1 receptor, did not differ between iron-deficient and control rats. In contrast, the number of D_2 receptors, the binding sites identified by using the radioligand 3H -spiroperidol, was significantly reduced. These results suggest that iron is either directly involved in the biochemical and functional aspects of D_2 receptors or indirectly involved via other chemical processes that serve to maintain the proper functioning or synthesis of these receptors. That such changes are absent in rats with a severe induced hemolytic anemia has been interpreted to mean that the behavioral alterations are due to iron deficiency rather than the associated anemia.^{20, 24} Experiments using transfusion to correct the anemia induced by iron deficiency but not the iron deficiency itself might be helpful in understanding the pathophysiologic changes underlying the observed behavioral alterations.

Even though the exact mechanism by which nutritional iron maintains D_2 receptor function is not yet understood, it connects research on iron deficiency to that on changes in the dopaminergic system in a variety of other conditions.

Dopamine may not be the only central nervous system biogenic amine affected by iron deficiency. Although altered dopaminergic transmission may ultimately provide the explanation,²⁵ behavioral alterations have been observed in the iron-deficient laboratory animal, which are not completely understood biochemically. For instance, there may be changes in several circadian rhythms in the iron-deficient animal. Some have observed a reversal in the spontaneous day/night patterns of activity, eating, and drinking in the iron-deficient rat, with more activity in the daytime instead of the normal nocturnal increase^{21, 26}; however, others have found the normal circadian pattern of nocturnal activity.²⁷⁻²⁹ Another example is the finding of elevated pain threshold and reversal of normal circadian changes in pain threshold following administration of β -endorphin, morphine, or haloperidol to iron-deficient rats.³⁰ Furthermore, biochemical changes that might explain the differences in learning in iron-deficient rats^{8, 31-36} have yet to be established. Even though dopamine seems to play a role in cognitive function^{13, 16} and dopaminergic transmission is affected by iron deficiency, the complexity of mechanisms underlying altered learning is suggested by the observations of Ruiz et al.³⁷ that early iron deficiency impairs the responsiveness of the parietal association area of the cerebral cortex.

Alterations related to serotonin, a neurotransmitter whose function is less well understood than that of dopamine, have also been reported: uptake of serotonin by synaptic vesicles was lower in the brains of iron deficient rats than controls.³⁸ Because the dopaminergic system is involved in the production of serotonin-induced behaviors,³⁹ changes in dopamine activity may also affect serotonin. Nevertheless, further studies may indicate that

behavioral changes in the iron-deficient animal relate to serotonin receptor function as well as dopamine receptor function. In addition, iron seems to play a role in the utilization of γ -aminobutyric acid (GABA),^{40, 41} a neurotransmitter, and the distribution of iron overlaps with enkephalin and luteinizing hormone-releasing hormone as well as that of GABA.⁴ Research on neurotransmitters is unfolding; the next decade may bring evidence of still other changes induced by iron deficiency or iron excess and lead to further clarification of iron's role in the central nervous system.

Altered behavior in iron deficiency may be due to changes in the periphery as well as in the central nervous system. Recent comprehensive reviews of the systemic biochemical changes² and changes in activity^{42, 43} induced by iron deficiency are available. Skeletal muscle is severely affected by iron deficiency. Myoglobin, which serves as oxygen transport and storage function in muscle, is decreased, and the iron-containing electron transport enzymes of the mitochondria are depleted. These changes seem functionally important: impairments in the capacity of the iron-deficient animal or human for sustained exercise have been consistently observed.⁴⁴⁻⁵⁴ Since changes in muscle function have been produced in isolated and perfused rat limb muscles,⁵⁵ a peripheral effect is involved. Yet the changes in dopaminergic neurotransmission produced by iron deficiency are also associated with alterations in movement. The relative importance of central and peripheral mechanisms in accounting for diminished work capacity and altered activity patterns has still to be determined. This illustration may serve as a reminder that a narrow focus on central nervous system biochemistry may prematurely limit understanding of the mechanisms producing behavioral changes in iron deficiency.

Recent Human Studies of Iron Deficiency and Behavior

Demonstrating an effect of iron deficiency anemia on human behavior has presented substantial methodologic challenges. To provide convincing proof of such an effect, a study should document clinically important behavioral differences between iron-deficient anemic subjects and nonanemic controls and demonstrate an improvement in behavior after iron therapy. The subjects should be similar in all respects except their iron status, and the treatment component should consist of a double-blind randomized trial of iron therapy with appropriate placebo controls that do not unduly delay specific therapy. Replication of results is essential. If the research addresses the related question of behavioral effects of iron deficiency that are independent of anemia, an additional set of methodologic considerations apply, such as the definition of iron status in nonanemic subjects and the timing of posttreatment assessments to precede any substantial increase in hemoglobin concentration.

Despite major methodologic strengths, many studies have been limited either by inadequate characterization of subjects' iron status, the lack of

appropriate normal controls, the omission of a placebo-treated group, or the failure to demonstrate changes in behavior following iron therapy. A number of studies in the last few years have yielded results that are similar enough to help offset methodologic shortcomings in any given study and to support some tentative conclusions about behavioral aspects of iron deficiency.

Characterization of Iron Status Among Humans

In animal studies there has been certainty about the animals' iron status. Iron deficiency can be experimentally induced by feeding the animals iron-deficient diets, and iron deficiency can be confirmed by tissue iron measurements as well as changes in the blood. These techniques are generally not possible with humans. Therefore, investigators use other methods of determining the iron status of human subjects. Since no single laboratory measure adequately characterizes an individual's iron status, most recent behavioral research reports have used multiple measures. The terms "iron replete" or "iron sufficient" have been used to denote a normal total body iron content.

Reductions in total body iron have been grouped into three stages of progressively increasing severity, based on current understanding of iron metabolism and defined by appropriate laboratory criteria.¹ Serum ferritin concentration, transferrin saturation, free erythrocyte protoporphyrin, and mean corpuscular volume are the tests most widely used in addition to hemoglobin or hematocrit. The term "iron depletion" has been used to designate a decrease in body storage iron without any effect on hemoglobin iron or iron in other functional iron compounds. This stage of iron deficiency is usually reflected by a decrease in the serum ferritin concentration without changes in other measures of iron status. The terms "iron-restricted erythropoiesis" or "iron deficiency without anemia" are used to describe an additional decrease in the iron supply such that heme production is limited, even though the resulting depression in the hemoglobin concentration or hematocrit is too slight to be detected using reference limits derived from population studies. In addition to a low serum ferritin, transport iron is decreased with a reduction in the serum transferrin saturation. Because insufficient iron is available to combine with protoporphyrin to form heme, protoporphyrin accumulates in the red blood cells and the free erythrocyte protoporphyrin increases. "Iron deficiency anemia" designates the anemia resulting from a further diminution in total body iron. The hemoglobin concentration is below reference limits, the serum ferritin and transferrin saturation decreased, and the free erythrocyte protoporphyrin elevated. The mean corpuscular volume decreases at about the same time the anemia becomes manifest.

Although a variety of confounding factors may distort this idealized pattern of changes in laboratory test results,⁵⁶ the use of multiple iron measures in recent behavioral studies represents an advance over earlier work.

Unless specifically indicated, the studies reviewed used at least three measures of iron status in addition to hemoglobin or hematocrit. A few studies have also included hematologic response to iron therapy as a criterion for defining iron deficiency. Because such a response is the most definitive of available standards, these studies will be identified.

Studies of Infants

Iron deficiency is most prevalent in the 6- to 24-month-old period, which coincides with the latter part of the brain growth spurt and with the unfolding of fundamental mental and motor processes. Infancy is an especially important age, therefore, in which to study behavioral effects of iron deficiency. In view of the potential clinical significance, 12 recent studies have focused on the behavior and development of iron-deficient infants. The major outcome measure in 10 of the 12 studies has been performance on the Bayley Scales of Infant Development,⁵⁷ a standardized test of infant development with three components: Mental Scale, Motor Scale, and Infant Behavior Record. In addition, a few projects have assessed infant behavior on other cognitive tests or outside the test situation. A strength of most of these studies is their inclusion of a therapeutic trial of iron, since improvement with iron treatment provides the most convincing evidence that the behavioral changes observed among iron-deficient infants are due to iron deficiency rather than to lack of another nutrient or to an intervening factor, such as disadvantaged home environment or limited parental intelligence. Taken as a group, these studies have been designed not only to establish whether behavioral alterations are present among iron-deficient infants, but also to address three further questions, to be considered in turn: (1) Does iron therapy produce rapid changes in behavior? (2) What is the degree of iron deficiency at which infant behavior is altered? (3) Does iron therapy completely correct these behavioral alterations?

In cases in which a given study addresses several of these questions, rather than presenting each study in its entirety, those components relevant to a particular question will be reviewed. This approach was also taken in another recent review to facilitate comparisons between studies,⁵⁸ but other sources consider each study separately.^{43, 59, 60}

Rapid Change in Behavior With Iron Therapy

Until the last 2 or 3 years, most studies examining the behavioral effects of iron deficiency were designed to detect changes in developmental test performance within 5 to 11 days of starting iron therapy. This emphasis on short-term treatment effects was guided by two considerations: (1) clinicians, in describing iron deficient anemic babies as irritable, apathetic, and distractible, have commented that these characteristics seem to disappear within a few days of iron treatment⁴³ and (2) early retesting might

allow any behavioral changes to be attributed to brain rather than blood, i.e., to altered central nervous system function rather than to the correction of anemia.

In Oski and Honig's pilot study, published in 1978,^{61, 62} 24 9- to 26-month-old iron-deficient anemic infants were randomly assigned to an intramuscular iron or placebo (saline injection) treatment group. A non-anemic control group was not included. One week after treatment, the Bayley mental test scores of anemic infants receiving intramuscular iron showed a significant mean increase of 14 points, while placebo-treated anemic infants had a nonsignificant mean increase of 6 points. These results were interpreted to "support the hypothesis that iron deficiency in infants produces developmental alterations and that these changes are rapidly reversible with iron therapy."⁶¹ However, the findings were equivocal. As Oski and Honig point out, the test-retest difference in mental test scores between the iron- and placebo-treated groups (14 vs. 6 points) was not statistically significant. Thus, although the increase in the iron-treated group's test scores was significant, the study did not demonstrate a clear effect of iron treatment over and above the effect of repeating the same test within a short period of time. Nonetheless, these results were intriguing and stimulated a number of related investigations.

In a study of 6- to 24-month-old Guatemalan infants, Lozoff et al.⁶³ compared 28 infants with iron deficiency anemia with a nonanemic group of 40 infants and assessed the effects of oral iron, the therapy of choice, and of placebo treatment. No changes due to short-term oral iron treatment were noted. Iron-treated anemic infants did not show significantly greater increases in their Bayley mental test scores (+7 points) than either placebo-treated anemic babies (+6 points) or infants in the two non-anemic groups (iron treated, +6 points; placebo treated, +5 points). The motor test performances of iron-treated anemic infants also did not improve more than that of infants in the other groups.

Subsequently, both Walter et al.,⁶⁴ using oral iron, and Oski et al.,⁶⁵ in a second study using parenteral iron, found short-term improvements among iron-deficient infants. However, neither study included placebo-treated control groups. The project by Walter et al.⁶⁴ in Chile involved 37 15-month-old infants. Ten iron-deficient anemic infants showed a significant increase of 10 points in Bayley mental test scores after 11 days of oral iron therapy, compared with a decrease of 1 point in the 12 iron-replete control infants. The scores of six iron-deficient infants whose hemoglobin levels were 11 gm/dl or higher also increased by 10 points, while those of nine iron-depleted infants increased by only 2 points. The second study by Oski and colleagues,⁶⁵ involving infants with normal hemoglobin levels (>11 gm/dl), demonstrated a dramatic increase in the mental scores of the 18 nonanemic iron-deficient infants one week after intramuscular iron. The 22-point increase in their mean scores was unambiguously greater than the 6-point change observed among the ten iron-depleted and ten iron-sufficient infants.

The results of these two studies^{64, 65} were interpreted as indicating that mental developmental test performance improved rapidly after iron therapy among iron-deficient infants, regardless of whether anemia was present. Because neither study included a placebo group, however, it is necessary to use caution in accepting this interpretation. It is not the improvements in test scores that are in doubt—the increases in the iron-deficient groups were both clinically and statistically significant—but the certainty with which these improvements can be attributed to iron therapy. In the absence of a placebo treatment, it cannot be determined whether the increases in test scores were due to iron therapy or to another factor that made the iron-deficient infants react differently than the control groups to the test-retest experience.

The apparent discrepancies in the results of some of the studies suggested that oral and intramuscular iron might differ in their short-term effects on developmental test scores. The effects of these two modes of treatment were therefore compared in a new study of 191 Costa Rican infants by Lozoff et al.⁶⁶ After 1 week of treatment, the increases in Bayley test scores and hematologic parameters among iron-deficient infants receiving intramuscular iron did not differ from those of iron-deficient infants receiving oral iron. The therapeutic modalities were therefore combined for comparison with placebo treatment. After 1 week, there was a significant increase in mental test scores regardless of whether the infants were anemic and treated with iron (+6 points) or placebo (+2 points) or nonanemic and treated with iron (+4 points) or placebo (+6 points). Similarly, there was no short-term effect of iron treatment on motor test scores.

One other study has assessed the effects of intramuscular iron. Moffatt and colleagues in Canada (personal communication, 1987) evaluated 34 6- to 24-month-old infants with iron deficiency anemia in a double-blind randomized controlled study in which half the children received intramuscular iron and half were given a sham injection. The Bayley scales were administered before treatment, after 1 week, and after 2 months. After 1 week, all infants were given oral iron for 2 months. A nonanemic control was not included in the study's design. Upon retesting at 1 week, mental scores increased an average of 4 to 5 points in both the iron-treated and placebo groups, and the effect of treatment on motor scores was similarly nonsignificant.

A new study by Walter and colleagues in Chile⁶⁷ also failed to reveal rapid improvements in test scores after oral iron therapy. Short-term increases in test scores were observed regardless of the treatment the infants received or their iron status prior to treatment. After receiving oral ferrous sulphate or placebo for 10 to 12 days, infants in the iron-deficient anemic, iron-deficient nonanemic, and control groups showed improvement in mental and motor scores, with increases averaging 4 to 9 points.

Though the number of available studies is still limited, consistent results have been obtained in all five that included a placebo treatment: the investigations in Guatemala⁶³ and Costa Rica,⁶⁶ Oski and Honig's original

study,⁶¹ the Moffatt et al project in Canada (personal communication), and the second Walter et al study.⁶⁷ Together, these studies indicate that short-term increases in test scores observed among iron-treated anemic infants are not significantly greater than those among placebo-treated anemic infants; increases in scores were observed regardless of the treatment the infants received or their iron status prior to treatment. The results of these studies indicate that an increase in Bayley test scores can be expected if the Bayley scales are readministered after a short time period and that these improvements probably reflect the effect of practice, since they cannot be attributed to iron therapy.

In research with the rat, by contrast, rapid behavioral changes have frequently been reported. Explanations for this disparity might be that the behaviors assessed in the rat and human studies are not comparable and/or that the iron deficiency did not occur at comparable points in development for the two different species. Recent studies by Ben-Shachar et al.¹¹ and Ruiz et al.³⁷ provide support for these explanations: learning deficits and changes in the reactivity of the parietal association cortex that were not rapidly corrected by treatment were noted when iron deficiency anemia was induced in newborn rats, whereas altered activity patterns have quickly returned to normal in other studies using older animal.^{10, 19}

Studies of short-term effects of iron therapy among human infants have also left two other important issues unsettled: the degree of iron deficiency that impairs infant behavior and the effectiveness of iron therapy in completely correcting behavioral alterations.

Degree of Iron Deficiency at Which Infant Behavior Is Adversely Affected

Oski and Honig's pilot study⁶¹ and the recent study by Moffatt et al in Canada (personal communication, 1987) did not directly address this issue because the studies did not include a nonanemic comparison group. However, three studies have compared the developmental test performance of infants with iron deficiency anemia to that of appropriate nonanemic controls. In the Guatemalan study described in the preceding section,⁶³ the mean mental development test score of the 28 iron-deficient anemic infants was 87 compared with the mean score in the nonanemic group of 100; a 9-point pretreatment difference in motor scores was also observed. Mental test score deficits were especially marked in older anemic infants, and the pattern of item failure suggested particular difficulty with verbal items.⁶⁸ A significant correlation between the degree of iron deficiency and mental test scores was also observed in the older age group.⁶⁸ In the first Chilean investigation by Walter et al.,⁶⁴ the average mental test score of ten iron-deficient anemic babies was 98, 12 points less than the mean score of the 27 infants with hemoglobin levels greater than or equal to 11 gm/dl; there was no difference between groups in motor scores. Somewhat similar results were also obtained in a recent study of 145 Asian 21- to 23-

month-olds living in the United Kingdom.⁶⁹ Sixty-one children with hemoglobin levels less than 11 gm/dl received lower scores on a developmental screening test than children with higher hemoglobin levels; significant between-group differences were noted in the areas of fine motor and social development. Whether there was a particular level of anemia at which developmental test performance was adversely affected was not specifically addressed by these studies, however.

Only one previous study in which iron status was carefully determined focused solely on infants with normal hemoglobin levels but varying degrees of iron lack. Oski et al.,⁶⁵ in a study briefly described previously, examined 38 9- to 12-month-old infants with hemoglobin levels greater than 11 gm/dl. Eighteen infants were iron deficient without anemia, ten were iron depleted, and ten were iron sufficient. The average mental test score of the infants with iron deficiency was 85 points, 10 points lower than those with iron depletion. However, the scores of the completely iron replete infants (mean = 91) fell between those of the iron-deficient and iron-depleted babies. Thus, using suitable iron-replete controls, the study did not find a statistically significant test score deficit in the iron-deficient but nonanemic group.

Differences in Bayley test scores were not obtained in two studies in which the measures of iron status were more limited. Johnson and McGowan,⁷⁰ drawing a sample of 1-year-old infants from a low income Mexican-American population, found no differences in mental or motor test scores between 25 anemic infants (hemoglobin levels <10.5 gm/dl) and an equal number of matched nonanemic controls. Iron deficiency was presumed to be the cause of the anemic infants' low hemoglobin levels; however, no measures of iron status were obtained. Deinard et al.,⁷¹ in a study of 9- to 11-month-old infants, all of whom had hematocrit levels greater than or equal to 34%, did not find cognitive test score differences among either a group of 34 infants with low ferritin levels, a second group of 21 babies with intermediate ferritin values, or a third group of 157 babies with higher ferritin values. Because the serum ferritin was the only available measure of iron status, however, infants with iron depletion could not be distinguished from those with iron deficiency.

It cannot be determined from these studies whether iron deficiency in the absence of anemia truly is not associated with developmental test score deficits or whether the lack of statistical significance was due to some methodologic limitation, such as small sample size or ambiguity in iron status. The lower scores of the infants in the iron-deficient nonanemic group in the Oski et al study,⁶⁵ though not statistically significant, and the observed relationship between degree of iron deficiency and mental scores among older infants in the Guatemalan study⁶⁸ suggested that further research was needed to resolve the issue of the *degree* of iron deficiency at which infant behavior and development become affected. The recent study in Costa Rica by Lozoff et al.⁶⁶ therefore addressed this issue by enrolling in a single study a relatively large number of infants with varied iron status.

The sample consisted of 191 12- to 23-month-old infants divided into groups ranging from most to least iron deficient as follows: (1) iron-deficient anemic ($n=52$), (2) intermediate in hemoglobin level and iron deficient ($n=45$), (3) nonanemic iron deficient ($n=21$), (4) nonanemic iron depleted ($n=38$), and (5) nonanemic iron sufficient ($n=35$). The data of the anemic infants were further analyzed with respect to actual hemoglobin level, since lower hemoglobin levels indicate more severe iron deficiency once anemia is present.

Infants with moderate iron deficiency anemia (hemoglobin ≤ 10.0 gm/dl) were found to have lower mental and motor test scores than appropriate controls; infants with mild anemia (hemoglobin = 10.1 to 10.5 gm/dl) received lower motor scores but not mental scores; and infants with lesser degrees of iron deficiency did not have impairments in developmental test performance. The mean mental test score of the moderately anemic infants was 8 points below that of infants with higher hemoglobin levels (>10.0 gm/dl), and the mean motor score of the entire anemic group was 10 points below that of infants with hemoglobin levels greater than 10.5 gm/dl. Mental test scores decreased with age in all groups; the differential decrease observed among older anemic infants in the Guatemalan study was not found.⁶⁸ However, anemic infants in all age groups seemed to have trouble with particular motor functions involving balance and coordination.

The results of the Costa Rican project are noteworthy for several reasons. The study was community based, thus minimizing biases potentially involved in research with patient populations. Infants with all known risk factors for altered hematologic or developmental status had been carefully excluded. Iron-deficient and iron-depleted conditions were confirmed by hematologic response to iron therapy. Finally, an extensive set of background variables relating to birth, general nutritional status, lead level, family background, home environment, and parental IQ failed to reveal any factor other than iron deficiency anemia that might explain the findings.

Similar results were obtained in another study with a strong research design, recently completed by Walter and associates in Chile.⁶⁷ This project is exceptional because the infants were studied prospectively from birth as part of a field trial of iron-fortified foods. Participating infants in an entire community were randomly assigned iron fortification or control. This feature of the study is especially important, since the disadvantages in the home environment that may be associated with iron deficiency were largely controlled by the random allocation procedure. Iron measures were obtained at 9 and 12 months and developmental assessments initially performed at 12 months. Not surprisingly, most of the iron deficiency and anemia occurred among the infants who did not receive iron-fortified foods. As in the Costa Rican study, iron status was ultimately confirmed by hematologic response to therapeutic iron, administered after 12 months of age. The developmental assessments at 12 months indicated that the 39 infants with iron-deficiency anemia had significantly lower mental and

motor scores than either 127 iron-deficient nonanemic infants or 30 iron-replete controls. The differences were similar to those obtained in Costa Rica: mental scores of the anemic infants averaged 6 to 7 points lower than those of nonanemic infants, motor scores averaged 9 to 11 points lower, and test score differences were not observed in the absence of iron deficiency anemia. An analysis of specific items on the mental scale suggested that the anemic infants had particular difficulty with verbal items, as observed in the study in Guatemala.⁶⁸ On the motor test, anemic infants had difficulty with items similar to those reported in the recent study in Costa Rica.⁶⁶

Due to its prospective design, the new study by Walter et al.⁶⁷ provides insight into the importance of chronicity and severity in iron deficiency anemia. Those infants who were anemic at both 9 and 12 months had significantly lower developmental test scores than those with anemia of less than 3 months' duration (i.e., those who had normal hemoglobin levels at 9 months and anemic levels at 12 months). As would be expected by the pathophysiology of iron deficiency, infants who were anemic at 9 and 12 months of age had lower hemoglobin levels than those whose anemia was not apparent until the 12-month testing. Although results of other studies have suggested a need for considering the chronicity, severity, and timing of iron deficiency, the results of this project, unique among published studies on the behavioral effects of iron deficiency for its prospective design, confirm the importance of such factors.

In sum, the results of research published to date support the conclusion that iron deficiency severe enough to cause anemia is associated with impaired performance on developmental tests in infancy. Five published studies with careful definition of iron status and nonanemic control groups, conducted in Guatemala,⁶³ Chile,^{64, 67} the United Kingdom,⁶⁹ and Costa Rica,⁶⁶ found clinically and statistically significant lower mental test scores among anemic infants prior to treatment. Lower motor test scores among anemic infants were also noted in the studies in Guatemala,⁶³ Costa Rica,⁶⁶ and the second project by Walter et al. in Chile.⁶⁷ The alterations in motor test performance are particularly interesting in view of the consistent changes in motor activity in the iron-deficient laboratory animal and the interrelatedness of locomotor function and cognitive development in the human infant. Significant cognitive or motor deficits have not yet been found among nonanemic infants with varying degrees of iron lack, but it would be premature to conclude that there are no ill effects. Furthermore, although consistent, the results of several studies of anemic infants do not mean that the observed alterations are due to anemia per se rather than to iron deficiency. Iron deficiency anemia develops only after a relatively prolonged period of iron lack. Thus, in the absence of rapid behavioral changes preceding hematologic improvement, it is extremely difficult to separate the effects of anemia from those of iron deficiency. Experimental manipulations, such as transfusion to correct anemia without altering iron deficiency, would enable one to disentangle the two conditions. Since

these experiments are not appropriate in the human infant, the relative roles of anemia and iron deficiency are likely to remain ambiguous in the foreseeable future.

That lower mental and motor test scores have been observed among iron-deficient anemic infants raises the further questions of *why* their scores were lower and *how* they might improve rapidly. Recent investigations have often assumed that disturbances in affect, arousal, or attentiveness were important determinants of anemic infants' poorer developmental test performance.^{70, 72, 73} However, attempts to document such alterations have been hindered by the paucity of standardized measures of noncognitive behavior in infancy. Eight of the available studies reported assessments of noncognitive behavior by means of the ratings on the Bayley Infant Behavior Record, but the results are often not directly comparable due to the different analytic methods employed.⁷⁴ Other limitations, such as small sample size, restricted measures of iron status, and the absence of either placebo or nonanemic control groups, have already been noted. Nonetheless, a number of behavioral alterations, including changes after treatment and differences between iron-deficient and control groups, have been observed.

Oski and Honig⁶¹ reported that iron-deficient anemic infants became more alert and responsive and better coordinated after iron therapy. Walter et al.⁶⁴ noted that anemic infants were more likely to be rated as unhappy during initial developmental testing and to become more cooperative and attentive on retesting. Honig and Oski observed that iron-deficient infants with hemoglobin values greater than 11 gm/dl were less irritable, more persistent, easier to engage in play, and less solemn after treatment.^{65, 75} Although no treatment-associated changes in behavior ratings were reported in the Guatemalan study,^{76, 77} behavioral disturbances were evident among the iron-deficient anemic infants. Moreover, those disturbances were closely related to poor developmental test performance. Anemic infants who were unduly fearful, unhappy, tired, tense, and hesitant or withdrawn with the examiner received low mental test scores, whereas anemic infants who were rated normal in affect achieved mental test scores comparable with those of nonanemic controls and normal by US standards.^{76, 77} Anemic infants and their mothers also maintained closer contact during play than nonanemic infants and their mothers.⁷⁸ Walter et al.,⁶⁷ analyzing the Infant Behavior Record in ways similar to those reported by Lozoff et al.,^{74, 77} found a pattern of behavioral differences quite like those observed in Guatemala: lower mental scores were noted among anemic infants who were abnormal in affect or task orientation. On individual Infant Behavior Record ratings, anemic infants were less responsive to the examiner, their mothers, and people in general, unhappier, less goal directed, showed shorter spans of attention, vocalized less, and moved less. In addition to differences in behavioral ratings, these investigators observed a pattern of cardiac responses to auditory habituation indicative of

less advanced attentional processing among anemic infants (DeAndraca et al., personal communication, 1987). Related to these findings may be the results of Moffatt et al. (personal communication, 1987) suggesting that anemic infants' orientation to tasks may improve with iron therapy. Despite the absence of between-group differences in mental test scores in the study by Deinard et al.,⁷¹ several behavioral differences between infants with low ferritin values and controls were reported. Only one study, by Johnson and McGowan,⁷⁰ reported no behavioral differences. On balance, with seven of eight studies noting noncognitive behavioral differences between anemic and nonanemic infants during developmental testing or play or behavioral changes after treatment, the results indicate the fruitfulness of examining affect, attention, and activity as well as standard test scores.

Complete Correction of Behavioral Alterations With Iron Therapy

The early search for rapid behavioral changes was motivated by an interest in attributing improvements in behavior and in test scores to improved function of iron-dependent central nervous system enzymes rather than to the correction of anemia. Although separating the effects of iron deficiency from those of anemia is important, a more pertinent question from a clinical perspective is whether iron therapy *completely corrects* any behavioral abnormalities, regardless of how soon changes might be detectable. Until very recently, none of the infant studies could address this issue because none included assessments after a longer course of iron therapy.

The recent study in Costa Rica⁶⁶ was specifically designed to examine the effects of a course of treatment commonly used in practice—3 months of oral iron therapy. On the basis of hematologic response to iron therapy, infants who became iron sufficient by study conclusion were distinguished from those who did not correct all evidence of iron deficiency. Three months of iron therapy was sufficient to correct completely the iron deficiency of nine infants (26%) in the group that was initially moderately anemic, even though none of the others remained anemic. Lower mental test scores were no longer evident among these nine infants. However, the absence of a posttreatment difference was due *not* to significant improvements in the mental test scores of the formerly moderately anemic infants but to the slight but statistically significant decline in mental scores after 3 months in the comparison group. Those anemic infants who did not become iron sufficient concluded the study with mental scores that were still significantly lower (mean = 93.2) than those of infants with initial hemoglobin levels greater than 10.0 gm/dl, regardless of whether the latter were iron sufficient after 3 months (mean = 101.8) or not (mean = 100.2). In contrast to the pattern of mental test score results, previously anemic infants who became iron sufficient by study's end did show a substantial increase in *motor* test scores, averaging 10 points, while the motor scores

of infants with hemoglobin levels greater than 10.5 gm/dl who became iron sufficient remained approximately the same. Previously anemic infants who did not become iron sufficient concluded the study with motor scores (mean = 106.3) that were still substantially lower than those of infants with initial hemoglobin levels greater than 10.5 gm/dl (mean = 114.9). There was laboratory evidence that anemic infants who did not become iron sufficient after 3 months had more severe and chronic iron deficiency.

Similar results, indicating that the majority of anemic infants do not show improvement after iron therapy, have been obtained in two other studies, one in the United Kingdom⁷⁹ and the other in Chile.⁶⁷ (The study by Mofatt et al. [personal communication, 1987], despite follow-up after 2 months, does not address this issue, since all anemic children were treated with iron and there was no nonanemic control group). Aukett et al.⁷⁹ in a double-blind randomized study of 17- to 19-month-old iron-deficient children in the United Kingdom found that 58% of those who showed a distinct hematologic response to 2 months of therapeutic iron (hemoglobin increase ≥ 2 gm/dl) failed to show the rate of development expected for their age. The expected rate of development was defined as the number of new items on a developmental screening test that 50% of average children in this age group would be expected to pass over a 2-month interval. It is important to note, however, that a greater proportion of children showing a marked hematologic response to iron therapy did achieve the expected rate of development than those who were treated with iron but whose increase in hemoglobin level was less than 2 gm/dl. The use of placebo treatment for 2 months is a methodologically strong, though controversial, aspect of this study's design, but the results are difficult to compare to those of other studies for several reasons: the developmental measure was unlike that used in other projects; analyses of mean developmental scores revealed no significant effects of treatment; and the decision to consider an increase in hemoglobin of 2 gm/dl or more as indicating effective treatment has no counterpart in other studies and may be a somewhat arbitrary cut-off that separated the children in this particular study. Nonetheless, this study, in conjunction with the one in Costa Rica,⁶⁶ suggests that iron therapy may favorably affect developmental test scores among some anemic children, but not the majority. The only other study relevant to the question of longer-term iron therapy is the second study by Walter and associates in Chile,⁶⁷ which is directly comparable in design to that in Costa Rica. As in the latter study, the administration of oral iron was carefully supervised and an excellent hematologic response documented. However, in contrast to the results obtained in Costa Rica, even those anemic infants who corrected their hematologic status failed to improve their scores. Thus, no improvements in mental or motor test scores were observed after three months of treatment.

The lack of improvement with iron therapy in these studies makes it difficult to be certain that the lower scores are due to iron deficiency ane-

mia, rather than some other nutrient or environmental deprivation. Although several investigators have seriously grappled with this possibility by exhaustive attempts to measure and control potentially intervening factors, the possibility that iron deficiency anemia is acting as a marker for other underlying problems must be kept in mind.

Inasmuch as the follow-up period in the Costa Rican, Chilean, and British studies was only 2 to 3 months, these studies cannot determine whether ill effects of iron deficiency anemia persist beyond infancy. The lower mental and motor test scores among many of the iron-deficient anemic infants might have responded to a more extended course of iron therapy. It is also possible that these differences might disappear spontaneously, especially because Bayley scores in the second year of life are only moderately correlated with measures of cognitive function in childhood.^{80, 81} Alternatively, the deficits might persist even if laboratory evidence of iron deficiency had been entirely corrected in all the anemic infants. This outcome would indicate that iron deficiency anemia in infancy, perhaps of a particular severity or chronicity, has irreversible ill effects.

Three observations support the latter worrisome possibility. First, in a series of studies with young rats, a brief period of induced iron deficiency anemia produced a deficit in brain iron and behavioral effects that persisted into adulthood despite correction of the associated anemia.^{7, 8, 11, 12, 32, 33, 37} Second, in a follow-up study of Israeli children treated for iron deficiency anemia in infancy, Palti et al.⁸² noted that lower hemoglobin levels at 9 months were associated with lower developmental and IQ test scores obtained as many as 4 years later. Even after controlling for other important factors, such as mother's education, social class, and birth weight, these investigators found a 1.75-point increase in IQ at age 5 with each 1 gm/dl increment in hemoglobin level. This effect on IQ scores is noteworthy because the infants' anemia had been diagnosed early and treated with iron as part of a health surveillance program. Although iron status was documented only by hemoglobin levels and hematologic response to iron treatment was not confirmed, the results suggest that anemia, as commonly diagnosed and treated in many parts of the world, may affect intellectual development even several years after it has been treated. In an abstract, Cantwell⁸³ described a preventive trial of iron therapy in which subjects were followed for 6 to 7 years. Children who had been anemic between 6 and 18 months of age ($n=32$) were less adept than children in whom anemia had been prevented ($n=29$) at balancing on one foot, tandem walking, and repetitive hand or foot movements. The IQ scores of the formerly anemic children were also 6 points lower. These concerns about lasting ill effects of iron deficiency anemia in infancy should be considered speculative. However, the results of the research to date would seem to warrant further research to assess the effectiveness of a prolonged course of iron therapy and to evaluate children who had iron deficiency anemia as infants.

Older Children, Adolescents, and Adults

Studies of behavioral changes in age periods after infancy are heterogeneous in terms of methodology and of the age groups examined. Although this diversity means that few results have yet been replicated, these studies have addressed two important questions that are methodologically difficult to study in infancy: what are the specific cognitive functions that may be impaired by iron deficiency and what is the functional significance of behavioral alterations due to lack of iron.

Several recent studies have assessed behavioral alterations in children, adolescents, and adults with varying degrees of iron lack and/or anemia. Seshadri et al. in India⁸⁴ reported improved IQ scores in anemic children after 2 months of an oral iron-folic acid supplement. This study, expanding on experience with a pilot project, included 14 5- to 6-year-old boys with hemoglobin levels between 8.0 and 10.5 gm/dl who received the supplement and a matched group of 14 anemic boys given placebo. After 60 days, the treated group had average increases of 10 to 17 points in verbal, performance, and total IQ scores, while the placebo group averaged 5- to 7-point increases. For verbal and total IQ, these increases were statistically significant in both the treatment and placebo groups, but performance IQ scores were significantly increased only among iron-treated boys. On all three scales the final scores of the treated group were significantly higher than those of the untreated group. Based on these results the authors concluded that iron-folic acid supplements improved intelligence test scores, particularly on nonverbal tasks. However, some features of the study's design limit the confidence with which the observed changes can be attributed to the treatment of iron deficiency. The children's iron status cannot be determined with certainty. Their anemia was presumably due to iron deficiency, since cell morphology on smear was hypochromic and microcytic and the treated group increased 2.4 gm/dl in hemoglobin concentration after 60 days. Such a response to treatment would ordinarily constitute proof of iron deficiency even without laboratory measures of iron status, but the addition of folic acid to the supplement introduces a confounding factor. The absence of nonanemic control group further limits interpretation, since it is not clear that the scores of the anemic children reflect a deficit compared to those of normal children.

In studies of 3- to 6-year-old children, one in the United States⁸⁵ and the other in Guatemala,⁸⁶ Pollitt and associates used a sophisticated test battery, developed from an information processing model, to assess attention, learning, and memory processes independently. In the US study,⁸⁵ the performance on this test battery of 15 "sideropenic" children was compared with that of another 15 children with normal iron status and matched to the sideropenic group with respect to age, sex, race, and height. On initial psychologic evaluation, the children whose transferrin saturations subsequently increased required more trials to reach a learning

criterion in three discrimination learning tasks and made more errors only in the simplest of the memory tests. The differences in the learning tasks disappeared on the second evaluation. Similar results were reported in a second study,⁸⁶ conducted in Guatemala, which compared 20 iron deficient anemic children whose hemoglobin levels increased after 11 to 12 weeks of oral iron treatment with 20 matched controls whose hemoglobin levels were at least 11.5 gm/dl before and after the same iron therapy. Pollitt et al. interpreted this pattern of results on the cognitive test battery to mean that improved iron status was associated with improved attentional processes, without effect on memory or rate of learning. However, aspects of the treatment component in each study hinders such an interpretation. In the first project the control group apparently received no treatment at all, and the treatment component was ultimately ignored since treatment only partially corresponded to hematologic response. In the second study, results have been presented for those subjects who received iron treatment, but not for those who received placebo. In addition, the functional significance of the observed cognitive changes is unknown. Nonetheless, these studies are strong in that the final criterion for iron deficiency was improved iron status and the measures of cognitive function were conceptually derived from a model of cognition.

Another study of preschool children, recently completed in Java by Pollitt and associates,⁸⁷ has been reported at national meetings but not yet published. The preschool children of 235 families associated with a particular tea plantation received either oral iron or placebo for 8 weeks. A battery of cognitive tests, including two two-choice discrimination learning tasks, four oddity learning tasks, and the Peabody picture vocabulary test, was administered before and after treatment. The children were classified on the basis of their iron status before treatment and the data analyzed accordingly. All groups of iron-treated children, regardless of initial classification, increased their hemoglobin levels significantly, which indicates that iron status was marginal in this population as a whole. Iron-treated children obtained higher Peabody picture vocabulary test scores on second testing than those receiving placebo, regardless of initial iron status. In contrast, several of the learning tasks showed a differential response among the anemic children. After treatment the anemic iron-treated children had the best performance of all the groups on the reversal component of the discrimination learning task and on two of the four oddity learning tasks. Detailed discussion of this study must await full presentation of the results; at that point, it will be important to consider how these results fit with the model of cognitive alterations previously proposed by Pollitt and colleagues.^{85, 86}

Recent studies of iron-deficient anemic infants raise the possibility that iron therapy may not completely correct behavioral disturbances. Two investigations involving older children also suggest that there is cause for concern. Deinard et al.⁸⁸ found that 18- to 60-month-old iron-deficient children, with or without anemia, did not show the improvements in men-

tal test scores observed in control subjects upon retesting at 3 and 6 months. The control group was also rated as more responsive to examiner and environment and more positive in emotional tone. Although hematologic response to iron was used to confirm iron deficiency, this study is difficult to interpret for several reasons: the iron-deficient children did not receive lower test scores prior to treatment; the age range of the subjects was large and the sample size modest so that analysis within age groups was impossible; and there is no standard for interpreting the increased test scores in the control group. Although short-term increases in mental scores have been reported, the degree of improvement due to practice or test comfort that might be expected after intervals of 3 to 6 months is not well described in young children.⁸⁸

Another recent project with a powerful study design raises similar concerns about the effectiveness of iron treatment. This study, by Soemantri et al. in Indonesia,⁸⁹ not only assessed the effects of 3 months of iron therapy but also included a measure—school achievement test scores—that permit one to interpret the functional significance of abnormalities observed in iron-deficient children. Three months of iron or placebo treatment was given to a group of 119 children, 78 of whom had iron deficiency anemia and 41 of whom were nonanemic. Prior to treatment the anemic children had significantly lower achievement test scores than the nonanemic group. After treatment, the iron-treated anemic children's test scores significantly improved over those of the placebo-treated anemic children. However, pretreatment test score differences remained; at the conclusion of the study the achievement test scores of anemic children, whether iron- or placebo-treated, were still lower than those of nonanemic controls.

Another recent study, by Gopaldas and associates in India,⁹⁰ noted substantial improvements in cognitive function among iron-treated anemic school boys. The cognitive function tests were visual recall (a test of memorizing capacity), digit span (a test of attention, short-term auditory memory, and auditory sequencing), mazes (a test of visual motor coordination and visual perception), and a clerical task (a test of discrimination and perception), which were summed to give an overall score. Two levels of iron therapy were compared with placebo treatment, with medication administered at school for 60 days in a double-blind randomized design. Although the study does not permit pretreatment comparisons between anemic and nonanemic children because all 48 subjects were matched for baseline scores in the cognitive function tests, this matching procedure clarifies the effects of treatment. Boys supplemented with iron at the higher dose (40 mg) showed significant improvements in all cognitive functions compared to the placebo group, while those supplemented with 30 mg of iron showed improvements less consistently. Further data analysis, comparing anemic and nonanemic boys within each treatment group, indicated that it was the anemic children whose cognitive function benefited from iron therapy.

The studies in Indonesia⁸⁹ and India,⁹⁰ together with a mathematical model based on data from the Philippines proposed by Popkin and Lim-Ybanez,⁹¹ suggest that iron deficiency anemia may be an important influence on school achievement, especially in developing countries and in disadvantaged populations elsewhere. Attempts to extrapolate the results to other cultures and to estimate the magnitude of the functional impairment must be made cautiously, however, because the studies used measures specifically designed for the culture under study. Although the failure to correct the deficits completely in the Indonesian study⁸⁹ leaves unresolved concerns about long-lasting or irreversible behavioral alterations, achievement test scores and cognitive function did improve considerably with iron therapy. The observed improvements underscore the value of treating iron deficiency anemia.

Research on the effect of iron deficiency on cognitive function in adolescence and adulthood is even more limited. In 1970, Elwood and Hughes⁹² reported that neither cognitive function nor subjective symptoms were improved among anemic pregnant women whose hemoglobin levels increased with iron therapy. In the years following the publication of this study, little research was apparently undertaken. Recently, however, a few studies have been published. Tucker and associates⁹³⁻⁹⁵ reported a series of experiments assessing the relationships among measures of iron status, cognitive performance, and quantitative EEG measures in normal university students. In the most recent study,⁹⁵ with a relatively large sample size, higher levels of serum ferritin were associated with greater activation of the left hemisphere relative to the right, greater verbal fluency, and poorer nonverbal auditory task performance. The results were interpreted to "suggest that body iron stores are relevant to specific neurophysiological processes supporting attention."⁹⁵ Foley et al.⁹⁶ also found evidence of specific cognitive effects of biochemical iron deficiency among university undergraduates. They noted small but statistically significant negative correlations between zinc protoporphyrin levels and three spatial tests (correlation coefficients ranging from -0.18 to -0.23). The importance of these observations and some of the methodologic approaches await further study, however, especially since the iron status of almost all the subjects would generally be considered in the normal range.

A recent study of adults by Groner et al.⁹⁷ examined 14- to 24-year-old women who came for prenatal care at or before 16 weeks' gestation. Those with hematocrit levels greater than 31% were randomly assigned either a vitamin supplement with iron or vitamins alone for 1 month. The iron-treated group showed significant improvements on some measures of short-term memory and attention span. The study was interpreted to indicate a beneficial effect of iron therapy. However, because the iron status of the two groups was similar at the conclusion of the study and none of the subjects showed biochemical evidence of iron deficiency before or after treatment, this interpretation may be problematic.

The only other recent study of adults involved a small group of patients

who were made chronically iron deficient, but not anemic, by repeated venesections to treat polycythemia vera.⁹⁸ The one symptom that seemed clearly attributable to iron deficiency was intense craving for ice in four of the seven patients.

The few available studies on adult cognitive function thus fail to provide much information about behavioral effects of iron deficiency. In contrast, there is considerable evidence from a variety of perspectives that iron deficiency anemia adversely affects work capacity and spontaneous activity in the adult. Evidence has steadily accumulated that iron deficiency anemia limits maximal physical performance.^{42, 99, 100} A diminished ability to perform brief, intense exercise tasks has been shown for anemia produced by phlebotomy⁴⁵ and for iron deficiency anemia, under both field and laboratory conditions.^{46-48, 50, 54} In addition, evidence that submaximal endurance is decreased has also been provided by field studies.^{51, 52} As might be expected from research on physical performance in animals,⁴⁴ both anemia itself and iron deficiency separate from anemia seem to contribute to adverse effects on exercise capacity.⁴⁷ Some researchers,⁵³ however, while agreeing that mild iron deficiency limits maximal exercise capacity, have doubted that such a restriction would influence the activity of most people, because even manual laborers seldom work near their maximum capacity.

Despite these doubts, the few studies to examine the effect of iron deficiency on spontaneous or voluntary activity in humans concluded that iron deficiency has significant functional consequences. Edgerton and colleagues⁵² examined the effect of iron treatment on the spontaneous activity of eight carefully matched pairs of women with iron deficiency anemia who were employed as tea pickers in Sri Lanka; one of each pair was treated with placebo and one with ferrous sulfate. This study did not compare the activity of iron-deficient and iron-replete individuals because a control group of nonanemic women was not included. Activity was measured using a small motion-sensitive device strapped to the ankle of the volunteer and recorded, along with the electrocardiogram, on 4- or 24-hour recorders. Compared with their placebo-treated matched controls, the women treated with iron were approximately 40% more active after 2 weeks of therapy, and 80% more active after 3 weeks. Over the 3-week treatment period, the hemoglobin concentration in women receiving iron increased from about 8 to 11 gm/dl. Similarly, evidence that iron deficiency anemia compromised worker production with concomitant economic loss was presented in research by Basta et al.,⁵¹ Popkin,¹⁰¹ and Pollitt et al.⁸⁷

It is especially important to understand the functional significance of adverse behavioral effects of iron deficiency because iron deficiency anemia is the most common nutritional disorder in the world. An estimated 20% of the adult men, 35% of adult women, and 40% of the children in the world are anemic, and over half of the anemia is thought to be due to iron deficiency.¹⁰² To estimate the functional significance of iron deficiency,

Levin¹⁰³ used the existing research base, albeit flawed and incomplete, in a benefit-cost analysis of programs to reduce anemia. The economic and social value of the additional work output and educational attainment that might be expected from correcting or preventing iron deficiency anemia was compared with the costs of iron supplementation or fortification of food with iron. Analyzing data from three different countries, Indonesia, Kenya, and Mexico, Levin concluded that the benefits substantially outweighed the costs. Assuming medium benefits and costs, the benefit-cost ratios ranged from 7 to 70 for iron fortification of food and from 4 to 38 for medicinal iron supplementation. These benefit-cost ratios are comparable to those of highly valued and widely accepted interventions, such as preventing iodine or vitamin A deficiency. The consequences of behavioral alterations in iron deficiency may thus become powerful considerations in public health policy as decisions are made about the allocation of scarce health care resources.

Summary

Cognitive Function

There is reasonably good evidence that mental and motor developmental test scores are lowered among infants with iron deficiency anemia. Although the research on cognitive function in iron-deficient older children and adults is sparse and diverse, it suggests that there may be alterations in attentional processes associated with iron deficiency. Iron therapy has not yet been shown effective in completely correcting the observed disturbances. Although some aspects of cognitive function seem to change with iron therapy, lower developmental, IQ, and achievement test scores have still been noted after treatment. The mechanisms explaining the variety of behavioral ill effects of iron-deficiency anemia in widely different age groups are still unknown. It is possible that they are due to changes in dopaminergic neurotransmission, but the biochemical bases are not yet completely understood.

Noncognitive Disturbances

A variety of noncognitive alterations during infant developmental testing has also been observed, including failure to respond to test stimuli, short attention span, unhappiness, increased fearfulness, withdrawal from the examiner, and increased body tension. Exploratory analyses suggest that such behavioral abnormalities may account for poor developmental test performance in infants with iron deficiency anemia. These studies indicate the fruitfulness of examining noncognitive aspects of behavior, such as affect, attention and activity, in addition to specific cognitive processes.

Activity and Work Capacity

There has been a steady accumulation of evidence that iron deficiency anemia limits maximal physical performance, submaximal endurance, and spontaneous activity in the adult, resulting in diminished work productivity with attendant economic losses. The relative importance of central and peripheral mechanisms underlying these effects, the extent to which anemia or iron deficiency separate from anemia is responsible, and the counterpart in infants and children remain to be established.

This review has examined recent evidence from research on central nervous system biochemistry and from human studies that iron deficiency adversely affects behavior by impairing cognitive function, producing non-cognitive disturbances, and limiting activity and work capacity. The body of research taken as a whole provides increasingly persuasive arguments for intensifying efforts to prevent and treat iron deficiency anemia.

References

1. Bothwell TH, Charlton RW, Cook JD, et al: *Iron Metabolism in Man*. Oxford, Blackwell Scientific Publications, 1979.
2. Dallman PR: Biochemical basis for the manifestations of iron deficiency. *Ann Rev Nutr* 1986; 6:13-40.
3. Hallgren B, Sourander P: The effect of age on the nonhaem iron in the human brain. *J Neurochem* 1958; 3:41-51.
4. Hill JM, Switzer RC III: The regional distribution and cellular localization of iron in the rat brain. *Neuroscience* 1984; 11:595-603.
5. Youdim MBH: Brain iron metabolism: Biochemical and behavioral aspects in relation to dopaminergic neurotransmission, in Lajath A (ed): *Handbook of Neurochemistry*. New York, Plenum Press, 1985, vol 10, pp 731-755.
6. Rajan KS, Colburn RW, Davis JM: Distribution of metal ions in the subcellular fractions of several rat brain areas. *Life Sci* 1976; 18:423-432.
7. Dallman PR, Siimes M, Manies EC: Brain iron: Persistent deficiency following short-term iron deprivation in the young rat. *Br J Haematol* 1975; 31:209-215.
8. Weinberg J, Levine S, Dallman PR: Long-term consequences of early iron deficiency in the rat. *Pharmacol Biochem Behav* 1979; 11:631-638.
9. Findlay E, Ng KT, Reid RL, et al: Developmental changes in body iron status following prolonged iron depletion in the rat. *Physiol Behav* 1981; 27:1097-1102.
10. Youdim MBH, Green AR, Bloomfield MR, et al: The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. *Neuropharmacology* 1980; 19:259-267.
11. Ben-Shachar D, Ashkenazi R, Youdim MBH: Long-term consequence of early iron-deficiency on dopaminergic neurotransmission in rats. *Int J Dev Neurosci* 1986; 4:81-88.
12. Dallman PR, Spirito RA: Brain iron in the rat: Extremely slow turnover in normal rats may explain long-lasting effects of early iron deficiency. *J Nutr* 1977; 107:1075-1081.

13. Iversen SD: Brain dopamine systems and behavior, in Iversen LI, Iversen SD, Snyder SH (eds): *Handbook of Psychopharmacology*. New York, Plenum Press, 1977, pp 333–384.
14. Kelly PH: Drug-induced motor behavior, in Iversen LL, Iversen SD, Snyder SH (eds): *Handbook of Psychopharmacology*. New York, Plenum Press, 1977, pp 295–331.
15. Pincus JH, Tucker GJ: *Behavioral Neurology*, ed 3. New York, Oxford University Press, 1985, pp 217–286.
16. Simon H, Scatton B, LeMoal M: Dopaminergic A10 neurones are involved in cognitive functions. *Nature* 1980; 286:150–151.
17. Youdim MBH, Woods HF, Mitchell BD, et al: Human platelet monoamine oxidase activity in iron deficiency anemia. *Clin Sci Mol Med* 1975; 48:289–295.
18. Mackler B, Person R, Miller LR, et al: Iron deficiency in the rat: Biochemical studies of brain metabolism. *Pediatr Res* 1978; 12:217–220.
19. Youdim MBH, Green AR: Biogenic monoamine metabolism and functional activity in iron-deficient rats: Behavioral correlates, in Porter R, Fitzsimons W (eds): *Iron Metabolism*. Ciba Foundation Symposium 51 (new series). New York, Elsevier North-Holland, Inc, 1977, pp 201–226.
20. Youdim MBH, Ben-Shachar D, Ashkenazi R, et al: Brain iron and dopamine receptor function. *Adv Biochem Psychopharmacol* 1983; 37:309–321.
21. Youdim MBH, Yehuda S, Ben-Uriah Y: Iron deficiency-induced circadian rhythm reversal in dopaminergic-mediated behaviours and thermoregulation in rats. *Eur J Pharmacol* 1981; 74:295–301.
22. Barkey RJ, Ben-Shachar D, Amit T, et al: Increased hepatic and reduced prostatic prolactin (PRL) binding in iron deficiency and during neuroleptic treatment: Correlation with changes in serum PRL and testosterone. *Eur J Pharmacol* 1985; 109:193–200.
23. Barkey RJ, Amit T, Ben-Shachar D, et al: Characterization of the hepatic prolactin receptors induced by chronic iron deficiency and neuroleptics. *Eur J Pharmacol* 1986; 122:259–267.
24. Ashkenazi R, Ben-Shachar D, Youdim MBH: Nutritional iron and dopamine binding sites in the rat brain. *Pharmacol Biochem Behav* 1982; 17:43–47.
25. Youdim MBH, Yehuda S: Iron deficiency induces reversal of dopamine dependent circadian cycles: Differential response to d-amphetamine and TRH. *Peptides* 1985; 6:851–855.
26. Glover J, Jacobs A: Activity pattern of iron-deficient rats. *Br Med J* 1972; 2:627–628.
27. Edgerton V, Bryant S, Gillespie C, et al: Iron deficiency anemia and physical performance and activity of rats. *J Nutr* 1972; 102:381–399.
28. Martin JC, Martin DC, Dillman E, et al: Effects of ambient temperature upon diurnal activity in nutritionally iron-deficient rats. *Bull Psychon Soc* 1980; 15:18–20.
29. Dallman PR, Refino CA, Dallman MF: The pituitary-adrenal response to stress in the iron-deficient rat. *J Nutr* 1984; 114:1747–1753.
30. Yehuda S, Youdim MBH: The increased opiate action of β -endorphin in iron-deficient rats: The possible involvement of dopamine. *Eur J Pharmacol* 1984; 104:245–251.
31. Findlay E, Reid RL, Ng KT, et al: The effect of iron deficiency during development on passive avoidance learning in the adult rat. *Physiol Behav* 1981; 27:1089–1096.

32. Weinberg J, Dallman PR, Levine S: Iron deficiency during early development in the rat: Behavioral and physiological consequences. *Pharmacol Biochem Behav* 1980; 12:493–502.
33. Williamson AM, Ng KT: Behavioral effects of iron deficiency in the adult rat. *Physiol Behav* 1980; 24:561–567.
34. Massaro TF, Widmayer P: The effect of iron deficiency on cognitive performance in the rat. *Am J Clin Nutr* 1981; 34:864–879.
35. Yehuda S, Youdim MBH, Mostofsky DI: Brain iron-deficiency causes reduced learning capacity in rats. *Pharmacol Biochem Behav* 1986; 25:141–144.
36. Youdim MBH, Ben-Shachar D: Minimal brain damage induced by early iron deficiency: Modified dopaminergic neurotransmission. *Israel J Med Sci* 1987; 23:19–25.
37. Ruiz S, Walter T, Perez H, et al: Effect of early iron deficiency on reactivity of the rat parietal association cortex. *Intern J Neurosci* 1984; 23:161–168.
38. Kaladhar M, Narasinga Rao BS: Effects of iron deficiency on serotonin uptake in vitro by rat brain synaptic vesicles. *J Neurochem* 1982; 38:1576–1581.
39. Green AR, Grahame-Smith DG: The role of brain dopamine in the hyperactivity syndrome produced by increased 5-hydroxytryptamine synthesis in rats. *Neuropharmacology* 1974; 13:949–959.
40. Hill JM: Iron concentration reduced in ventral pallidum, globus pallidus, and substantia nigra by GABA-transaminase inhibitor, gamma-vinyl GABA. *Brain Res* 1985; 342:18–25.
41. Gabrielsson B, Robson T, Norris D, et al: Effects of divalent metal ions on the uptake of glutamate and GABA from synaptosomal fractions. *Brain Res* 1986; 384:218–223.
42. Dallman PR: Iron deficiency: Distinguishing the effects of anemia from muscle iron deficiency on work performance, in Saltman P, Hegenauer J (eds): *The Biochemistry and Physiology of Iron*. New York, Elsevier North-Holland, Inc, 1982, pp 509–523.
43. Lozoff B, Brittenham GM: Behavioral aspects of iron deficiency. *Progr Hematol* 1985; 14:23–53.
44. Ohira Y, Edgerton VR, Gardner GW, et al: Work capacity after iron treatment as a function of hemoglobin and iron deficiency. *J Nutr Sci Vitaminol* 1981; 27:87–96.
45. Ekblom B, Goldbarg AN, Gullbring B: Response to exercise after blood loss and reinfusion. *J Appl Physiol* 1972; 33:175–180.
46. Davies CTM, Chukweumeka AC, van Haaren JPM: Iron deficiency anaemia: Its effect on maximum aerobic power and responses to exercise in African males aged 17–40 years. *Clin Sci* 1973; 44:555–562.
47. Gardner GW, Edgerton VR, Barnard RJ, et al: Cardiorespiratory, hematological, and the physical performance responses of anemic subjects to iron treatment. *Am J Clin Nutr* 1975; 28:982–988.
48. Gardner GW, Edgerton VR, Senewiratne B, et al: Physical work capacity and metabolic stress in subjects with iron deficiency anemia. *Am J Clin Nutr* 1977; 30:910–917.
49. Finch CA, Gollnick PD, Hlastala MP, et al: Lactic acidosis as a result of iron deficiency. *J Clin Invest* 1979; 64:129–137.
50. Viteri FE, Torun B: Anemia and physical work capacity. *Clin Haematol* 1974; 3:609–626.

51. Basta S, Soekirman KD, Scrimshaw NS: Iron deficiency anemia and the productivity of adult males in Indonesia. *Am J Clin Nutr* 1979; 32:916-925.
52. Edgerton VR, Gardner GW, Ohira Y, et al: Iron-deficiency anaemia and its effect on worker productivity and activity patterns. *Br Med J* 1979; 2:1546-1549.
53. Charlton RW, Derman D, Skikne B, et al: Anaemia, iron deficiency, and exercise: Extended studies in human subjects. *Clin Sci Mol Med* 1977; 53:537-541.
54. Ohira Y, Edgerton VR, Gardner GW, et al: Work capacity, heart rate, and blood lactate responses to iron treatment. *Br J Haematol* 1979; 41:365-372.
55. McLane JA, Fell RD, McKay RH, et al: Physiological and biochemical effects of iron deficiency on rat skeletal muscle. *Am J Physiol* 1981; 241: C47-C54.
56. Brittenham GM, Danish EH, Harris JW: Assessment of bone marrow and body iron stores: Old techniques and new technologies. *Semin Hematol* 1981; 18:194-221.
57. Bayley N: *Bayley Scales of Infant Development Manual*. New York, Psychological Corporation, 1969.
58. Lozoff B, Brittenham GM: Behavioral alterations in iron deficiency. *Hematol Oncol Clin N Am* 1987; 15:33.
59. Pollitt E, Saco-Pollitt C, Leibel L, et al: Iron deficiency and behavioral development in infants and preschool children. *Am J Clin Nutr* 1986; 43:555-565.
60. Pollitt E: Effects of iron deficiency on mental development: Methodological considerations and substantive findings, in Johnston FE (ed): *Nutritional Anthropology*. New York, Alan R Liss 1987, pp 225-254.
61. Oski FA, Honig AS: The effects of therapy on the developmental scores of iron-deficient infants. *J Pediatr* 1978; 92:21-25.
62. Honig AS, Oski FA: Developmental scores of iron deficient infants and the effects of therapy. *Infant Behav Dev* 1978; 1:168-176.
63. Lozoff B, Brittenham GM, Viteri FE, et al: The effects of short-term oral iron therapy on developmental deficits in iron deficient anemic infants. *J Pediatr* 1982; 100:351-357.
64. Walter T, Kovalskys J, Stekel A: Effect of mild iron deficiency on infant mental development scores. *J Pediatr* 1983; 102:519-522.
65. Oski FA, Honig AS, Helu B, et al: Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. *Pediatrics* 1983; 71:877-880.
66. Lozoff B, Brittenham GM, Wolf AW, et al: Iron deficiency anemia and iron therapy effects on infant developmental test performance. *Pediatrics* 1987; 79:981-995.
67. Walter T, deAndraca I, Chadud P, et al: Adverse effect of iron deficiency anemia on infant psychomotor development, submitted.
68. Lozoff B, Brittenham GM, Viteri FE, et al: Developmental deficits in iron-deficient infants: Effects of age and severity of iron lack. *J Pediatr* 1982; 101:948-952.
69. Grindulis H, Scott PH, Belton NR, et al: Combined deficiency of iron and vitamin D in Asian toddlers. *Arch Dis Child* 1986; 61:843-848.
70. Johnson DL, McGowan RJ: Anemia and infant behavior. *Nutr Behav* 1983; 1:185-192.
71. Deinard A, Gilbert A, Dodds M, et al: Iron deficiency and behavioral deficits. *Pediatrics* 1981; 68:828-833.

72. Pollitt E, Greenfield D, Leibel R: Significance of Bayley Scale score changes following iron therapy: II. *Infant Behav Dev* 1979; 2:235-238.
73. Honig AS, Oski FA: Reply to Pollitt et al. *Infant Behav Dev* 1979; 2:239-240.
74. Wolf AW, Lozoff B: A clinically interpretable method for analyzing the Bayley Infant Behavior Record. *J Pediatr Psychol* 1985; 10:199-214.
75. Honig AS, Oski FA: Solemnity: A clinical risk index for iron deficient infants. *Early Child Dev Care* 1984; 16:69-84.
76. Lozoff B, Brittenham G, Viteri FE, et al: Behavioral abnormalities in infants with iron deficiency anemia, in Pollitt E, Leibel RL (eds): *Iron Deficiency: Brain Biochemistry and Behavior*. New York, Raven Press, 1982, pp 183-194.
77. Lozoff B, Wolf AW, Urrutia JJ, et al: Abnormal behavior and low developmental test scores in iron-deficient anemic infants. *J Dev Behav Pediatr* 1985; 6:69-75.
78. Lozoff B, Klein NK, Prabucki KM: Iron-deficient anemic infants at play. *J Dev Behav Pediatr* 1986; 7:152-158.
79. Aukett MA, Parks YA, Scott PH, et al: Treatment with iron increases weight gain and psychomotor development. *Arch Dis Child* 1986; 61:849-857.
80. Kopp CB, McCall RB: Predicting later mental performance for normal, at-risk, and handicapped infants. *Life-Span Dev Behav* 1982; 4:33-61.
81. McCall RB: The development of intellectual functioning in infancy and the prediction of later IQ, in Osofsky JD (ed): *Handbook of Infant Development*. New York, John Wiley & Sons, 1979, pp 707-741.
82. Palti H, Pevsner B, Adler B: Does anemia in infancy affect achievement on developmental and intelligence tests? *Hum Biol* 1983; 55:189-194.
83. Cantwell RJ: The long-term neurological sequelae of anemia in infancy, abstract. *Pediatr Res* 1974; 8:342.
84. Seshadri S, Hirode K, Naik P, et al: Behavioural responses of young anaemic Indian children to iron-folic acid supplements. *Br J Nutr* 1982; 48:233-240.
85. Pollitt E, Leibel RL, Greenfield DB: Iron deficiency and cognitive test performance in preschool children. *Nutr Behav* 1983; 1:137-146.
86. Pollitt E, Viteri F, Saco-Pollitt C, et al: Behavioral effects of iron deficiency anemia in children, in Pollitt E, Leibel RL (eds): *Iron Deficiency: Brain Biochemistry and Behavior*. New York, Raven Press, 1982, pp 195-208.
87. Pollitt E, Scrimshaw NS, Soewondo V, et al: Functional consequences of iron deficiency anemia and nutrition planning in Indonesia. Paper presented at the Society for Research in Child Development Symposium on The Application of Research Findings to Development Programs: Examples for the Third World, Baltimore, Maryland, April 29, 1987.
88. Deinard AS, List A, Lindgren B, et al: Cognitive deficits in iron-deficient and iron-deficient anemic children. *J Pediatr* 1986; 108:681-689.
89. Soemantri AG, Pollitt E, Kim I: Iron deficiency anemia and educational achievement. *Am J Clin Nutr* 1985; 42:1221-1228.
90. Gopaldas T, Kale M, Bhardwaj P: Prophylactic iron supplementation for underprivileged school boys: II. Impact on selected tests of cognitive function. *Indian Pediatr* 1985; 22:737-743.
91. Popkin BM, Lim-Ybanez M: Nutrition and school achievement. *Sci Med* 1982; 16:53-61.
92. Elwood PC, Hughes D: Clinical trial of iron therapy on psychomotor function in anaemic women. *Br Med J* 1970; 3:254-255.

93. Tucker DM, Sandstead HH: Spectral electroencephalographic correlates of iron status: Tired blood revisited. *Physiol Behav* 1981; 26:439-449.
94. Tucker DM, Sandstead HH, Swenson RA, et al: Longitudinal study of brain function and depletion of iron stores in individual subjects. *Physiol Behav* 1982; 29:737-740.
95. Tucker DM, Sandstead HH, Penland JG, et al: Iron status and brain function: Serum ferritin levels associated with asymmetries of cortical electrophysiology and cognitive performance. *Am J Clin Nutr* 1984; 39:105-113.
96. Foley D, Hay DA, Mitchell RJ: Specific cognitive effects of mild iron deficiency and associations with blood polymorphisms in young adults. *Ann Hum Biol* 1986; 13:417-425.
97. Groner JA, Holtzman NA, Charney E, et al: A randomized trial of oral iron on tests of short-term memory and attention span in young pregnant women. *J Adol Health Care* 1986; 7:44-48.
98. Rector WG, Fortuin NJ, Conley CL: Non-hematologic effects of chronic iron deficiency: A study of patients with polycythemia vera treated solely with venesections. *Medicine* 1982; 61:382-389.
99. Jacobs A: The non-haematological effects of iron deficiency. *Clin Sci Mol Med* 1977; 53:105-109.
100. Scrimshaw NS: Functional consequences of iron deficiency in human populations. *J Nutr Sci Vitaminol* 1984; 30:47-63.
101. Popkin BM: Nutrition and labor productivity. *Sci Med* 1978; 12C:117-125.
102. DeMaeyer E, Adiels-Tegman M: The prevalence of anaemia in the world. *Rapp Trimest Statist Sanit Mond* 1985; 38:302-316.
103. Levin HM: A benefit-cost analysis of nutritional programs for anemia reduction. *Res Observ* 1986; 1:219-245.

Pathogenesis of the Anemia of Chronic Renal Failure: The Role of Erythropoietin

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In 1836, Richard Bright in his classic monograph on kidney disease¹ recognized the frequent occurrence of anemia in patients with renal failure. Since that time, significant progress has been made in the elucidation of the pathogenesis of the anemia of chronic renal failure (CRF). The anemia, however, remains a serious problem that limits the physical tolerance of many patients with CRF and prevents rehabilitation of patients receiving chronic dialysis treatments.²

In this chapter we review the role of the normal kidney in the regulation of erythropoiesis. We discuss the current concepts of the pathogenesis of the anemia of CRF with special emphasis on the role of erythropoietin (Ep) in the genesis and treatment of this anemia.

The Role of the Normal Kidney in the Regulation of Erythropoiesis

The role of the kidneys in regulating erythropoiesis was not recognized until 1957 when Jacobson and co-workers showed that removal of kid-

neys nearly abolished Ep production in rats.³ These investigators showed that the kidneys were the major source of Ep production.

Subsequent studies have shown that the normal isolated perfused kidney synthesizes Ep when perfused with a serum free medium,⁴ and that this synthesis is augmented when the kidney is perfused at low PO₂.⁵ Extracts from hypoxic kidneys have yielded Ep⁶ and Ep mRNA has been demonstrated in the kidney.⁷

Ep is considered to be the most important humoral regulator of erythropoiesis. Ep production is regulated by the supply of oxygen to the site(s) of its production relative to the oxygen requirement of this tissue.^{8, 9} The kidney serves a dual role in the regulation of red cell production; this organ is responsible for sensing oxygen availability to tissues as well as for releasing Ep into the circulation.⁹

Why the kidney should serve as the erythroid master organ may be related to the kidney's unique adjustment of oxygen demand to oxygen supply.¹⁰ Oxygen is consumed by the kidney primarily to fuel sodium reabsorption, which in turn depends on glomerular filtration rate (GFR) and renal blood flow. Thus, a reduction in oxygen supply due to decreased renal blood flow also decreases GFR and oxygen demand; this explains why decreased renal blood flow from multiple causes per se does not cause increased Ep production.¹⁰ However, renal ischemia beyond a critical degree does lead to increase in Ep production.¹¹

The Cell of Origin of Erythropoietin

The site of Ep generation within the kidney is a matter of debate. Renal tubular cells and juxtaglomerular apparatus have been considered as possible sites of renal Ep generation.^{12, 14} However, immunofluorescence studies with antisera produced against crude Ep have localized Ep in the glomeruli.¹⁵ Ep has been extracted from isolated glomeruli of hypoxic rats,^{16, 17} and cultured mesangial cells are capable of producing Ep in response to hypoxia.¹⁸

The liver has been identified as the primary site of Ep production during fetal life.¹⁹ However, after birth, Ep generation is shifted to the kidney and hepatic Ep contributes only 10% to 15% of total Ep in normal adults.²⁰ In the liver, Ep may be generated in the Kupffer cells. Ep immunofluorescent staining²¹ and isolated Kupffer cells are capable of generating Ep.²² Extra-renal Ep production has been found to increase in association with increase in Kupffer cell function,²³ in association with liver regeneration,²⁴ and by injection of either renin or angiotensin II.⁸

Recently, complementary DNA (cDNA) sequence has been cloned for Ep from human fetal liver²⁵ as well as human renal cell carcinoma.²⁶ The existence of Ep has also been demonstrated in salivary glands and macrophages.^{27, 28}

Function of Erythropoietin

Ep induces red cell formation by stimulating proliferation, downstream differentiation and maturation of erythroid progenitors and precursors.²⁹

Figure 1 provides a schematic diagram of the sites of Ep action in the bone marrow. Red cells are thought to originate from a primitive hematopoietic cell capable of producing progenitors of all types of blood cells. This stem cell has been termed the colony forming unit-spleen (CFU-S) because transplantation of these cells into spleens of heavily irradiated mice gives rise to erythroid, granulocytic and megakaryocytic colonies.³⁰ CFU-S has the unique capacity of nearly indefinite self replication. The mechanism by which CFU-S is committed to the erythrocytic pathway has

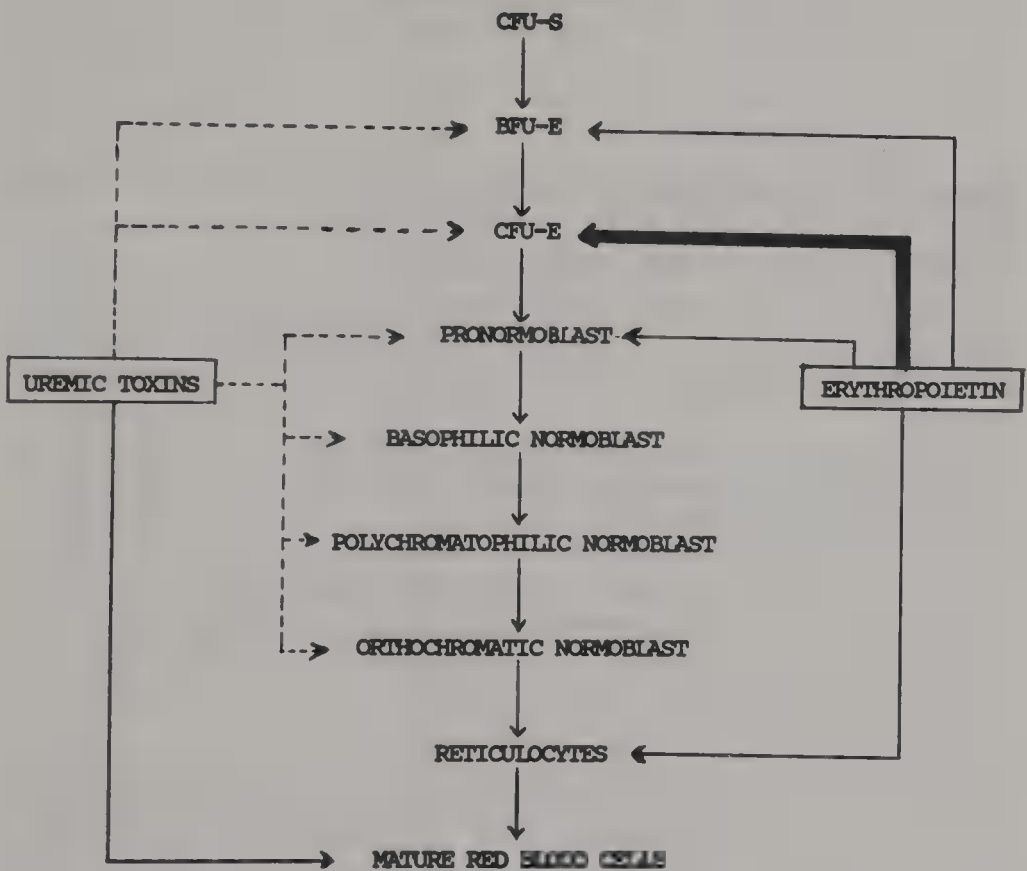


FIG 1.

Schematic diagram for the sites of action of Ep and uremic toxins during erythroid differentiation. The broken lines represent sites where uremic toxins inhibit erythropoiesis in *in vitro* studies; their role *in vivo* is not clear. The heavy line denotes exquisite sensitivity of the target cell to erythropoietin. CFU-S = colony forming unit-spleen; BFU-E = burst forming unit-erythroid; CFU-E = colony forming unit-erythroid.

not yet been clarified.³¹ The most primitive erythroid progenitor cell responding to Ep is called the burst forming unit-erythroid (BFU-E). Large doses of Ep are needed to induce BFU-E to produce multiclustered bursts of hemoglobin-synthesizing cells *in vitro*.^{32, 33} *In vivo*, the BFU-E are relatively insensitive to the alterations in the plasma Ep titer.^{32, 34} More sensitive and specific for Ep action is an erythroid progenitor cell called the colony forming unit-erythroid (CFU-E). In response to low concentration of Ep, CFU-E produces small clusters of hemoglobin-synthesizing cells.³⁵ CFU-E are similar to the basophilic normoblasts found in the bone marrow. Ep probably also stimulates early release of reticulocytes from the bone marrow.²⁹

In addition to the role of Ep in regulating erythropoiesis, lymphocytes, macrophages, and their conditioned media have been identified to influence proliferation and maturation of erythroid progenitors.^{28, 33, 36, 37} In addition, prostaglandins of E type, beta-adrenergic agonists, and certain androgenic steroids may also play a role in modulating erythropoiesis.³⁸⁻⁴⁰

Structure of Erythropoietin and Development of Biosynthetic Human Erythropoietin

In 1977, Miyake et al. isolated a relatively large quantity of Ep from the urine of severely anemic patients and purified it to apparent homogeneity.⁴¹ This material served as a source of pure Ep for the study of Ep structure.

Ep is a glycoprotein with a molecular weight of 34,000 daltons and contains approximately 25% carbohydrate, consisting mostly of sialic acid.⁴² The carbohydrate moiety is not critical for the erythropoietic action of Ep, but prevents its rapid-clearance.⁴³

In recent years, polyclonal and monoclonal antibodies were raised against purified Ep. This led to the development of radioimmunoassay (RIA) of Ep.⁴⁴ The most important contribution of the RIA was that it became possible to monitor the isolation and fractionation procedures needed to determine the amino acid sequence of Ep.⁴⁵ Part of this sequence was then used to predict the base composition of a corresponding cDNA, which in turn was used as a probe to identify the entire Ep gene.^{25, 26, 42} Molecular biologists have isolated the gene and inserted it into mammalian cells capable of synthesizing unlimited quantities of Ep.⁴⁶ It was necessary to use animal cells rather than bacteria because Ep, with its 166 amino acids, is highly glycosylated and only animal cells provide the necessary sugar components.

The recombinant human Ep has been purified from the cell conditioned medium to greater than 98% homogeneity. It is reported to have a specific activity of 129,000 IU/mg of hormone.⁴⁶ Complete sequence analysis of highly purified human urinary Ep⁴² and recombinant human Ep showed

that the two hormones have identical amino acid sequences, and on carbohydrate structure analysis they have similar oligosaccharide chains. In addition, the biological activity and immunological properties of the human urinary Ep and recombinant hormones have not been distinguishable.⁴⁶⁻⁴⁸

Clinical Characteristics of the Anemia of Chronic Renal Failure

The degree of anemia in patients with CRF is generally proportional to severity of uremia.^{49, 50} Severe anemia is generally not noted until the GFR falls below 20–40 ml/min/1.73m².^{49, 51} However, a wide scatter of hematocrit values is noted for the same level of renal function,^{51, 52} which reflects the multiple causes of renal failure as well as the multifactorial origin of the anemia.

After end stage renal disease has occurred, the degree of anemia generally remains relatively constant in patients maintained on hemodialysis or intermittent peritoneal dialysis. However, in patients treated with continuous ambulatory peritoneal dialysis (CAPD), a significant improvement in hematocrit is noted in the first year of CAPD treatment.^{53, 54} The anemia of chronic renal disease is generally milder in patients with polycystic kidney disease⁵⁵ and those with concurrent hypertension.⁵⁶ The anemia is more severe for the level of renal dysfunction in patients with medullary cystic disease, nephrotic syndrome, bilateral nephrectomies, and those with severe hyperparathyroidism.

Patients with CRF generally tolerate anemia better than do those with comparably severe anemias due to other causes. This may be related to the lower hemoglobin-oxygen affinity,⁵⁷ which results in a more effective delivery of oxygen to the tissues. In addition, diminution of renal blood flow allows a larger portion of the cardiac output to perfuse other tissues; under normal circumstances, approximately 20% of the cardiac output goes to the kidneys.^{58, 59} However, a substantial number of patients with CRF do suffer from decreased exercise tolerance and fatigue from the anemia.

Laboratory Characteristics of the Anemia of Chronic Renal Failure

The anemia of CRF, when uncomplicated by superimposed deficiency states or severe hemolysis, is characteristically normochromic, normocytic, and hypoproliferative.^{50, 60} The absolute reticulocyte count is low. The leukocyte and platelet counts are usually normal. The peripheral smear may show burr cells; their frequency is roughly proportional to the severity of uremia. This morphological abnormality is likely related to the change in red cell membrane secondary to alterations in the chemical composition of

the uremic plasma.⁶¹ Other morphological abnormalities of red blood cells in patients with CRF are seen in the following situations: megaloblastic changes in patients with folic acid deficiency, hypochromic microcytic cells in patients with iron deficiency or aluminum toxicity, schistocytes in patients with microangiopathic hemolytic anemia, spherocytes in patients with hypersplenism, acanthocytes and Howell jolly bodies in postsplenectomised patients, and cells with Heinz bodies in patients with abnormal red cell metabolism.

The bone marrow in patients with CRF is characteristically normocellular without abnormalities in maturation.^{60, 62} The number of erythroid precursors is usually normal or even slightly increased.⁶³ Ferrokinetic studies show decreased plasma iron turnover, decreased rate of red cell iron utilization, and increased marrow transit time.^{50, 60} In most patients, the pattern is that of erythropoiesis that is decreased relative to the severity of anemia although the red cell maturation is orderly.

Pathogenesis of the Anemia of Chronic Renal Failure

Table 1 lists the major pathogenetic mechanisms of the anemia of CRF, and Table 2 lists the factors that influence the severity of anemia. Ultimately the anemia results from a defect in the ability of the hematopoietic system to adequately respond to hypoxic stimuli. Thus, it cannot compensate for the relatively mild degree of hemolysis that occurs in patients with advanced renal failure. E_p deficiency appears to be the primary causative factor for the decreased erythropoiesis, although uremic inhibitors of erythropoiesis may also contribute to the problem.

TABLE 1.
Major Causes for the Anemia of Renal Failure

-
- | | |
|-----|---|
| I. | Decreased Erythropoiesis |
| A. | Decreased erythropoietin production |
| 1. | Loss of renal parenchyma |
| 2. | Decreased hemoglobin—oxygen affinity |
| 3. | Lowered set point for erythropoietin-tissue oxygenation-hematocrit feedback mechanism |
| B. | Inhibitors of cell proliferation and erythropoiesis |
| II. | Hemolysis due to effect of uremic toxins on RBC membrane and cell enzymes |
-

TABLE 2.
Factors That Influence the Severity of the
Anemia of Renal Failure

Situations associated with less severe anemia
Polycystic kidney disease
CAPD treatment
Hypertension
Liver cell necrosis and regeneration
Situations associated with increased severity of anemia
Decreased erythropoiesis
Bilateral nephrectomies
Nephrotic syndrome
Hyperparathyroidism
Medullary cystic disease
Concurrent infections
Deficiency states of iron, folic acid, histidine, protein
Aluminum toxicity
Osteitis fibrosa from secondary hyperparathyroidism
Exacerbation of hemolysis
Exposure of RBC to oxidant stress
"Stiff" cells due to hypophosphatemia
Microangiopathy
Hypersplenism
Overheated dialysate
Hypotonic dialysate
Contaminants in the dialysate
Excessive blood loss
Blood loss in the dialyser with hemodialysis
Gastrointestinal blood loss due to platelet defects
Blood drawing for laboratory testing
Surgical procedures

Decreased Red Cell Survival in Chronic Renal Failure

Decreased red cell survival is seen in all patients with advanced renal insufficiency using isotope red cell tagging with ^{51}Cr , ^{32}P , ^{14}C cyanate and measurement of carbon monoxide exhalation.^{64, 65} The red cell survival is shortened to approximately half normal.⁶⁴ Hemolysis, however, is mild enough that a normal hematopoietic system should be able to compensate for it. It is, therefore, not considered a major cause for the anemia of CRF. The hemolysis is caused by substances in uremic plasma that interfere with the RBC membrane's ability to effectively pump sodium from the cells.⁶⁶ The transfusion of normal red cells into uremic patients

results in a shortening of the survival of the transfused cells, while transfusion of red cells from patients with CRF into nonuremic subjects is associated with normal red cell survival.⁶⁷ Hemodialysis or CAPD does not significantly improve red cell survival.^{64, 68}

In selected patients with CRF, red cell survival may be further shortened from the following complications: (1) increased rigidity of the red cell membrane due to hypophosphatemia from overly aggressive treatment with phosphate binders⁶⁹; (2) microangiopathy from hypertension, vasculitis, or defective hemodialysis coils; and (3) hypersplenism from chronic antigenic stimulation⁷⁰ or from trapping of silicone particles that may have leaked from the dialysis tubing.⁷¹ Acute hemolysis has been observed if a patient's red cells are exposed during dialysis to various oxidants,⁷²⁻⁷⁴ formaldehyde,⁷⁵ or overheated⁷⁶ or hypotonic dialysate.⁷⁷ The function of the RBC pentose phosphate shunt enzyme is impaired in uremia,⁷⁸ hence these cells are more prone to the development of Heinz body hemolytic anemia in response to oxidant stress.

Inhibition of Erythropoiesis in Chronic Renal Failure

Several lines of evidence suggest that abnormal metabolites or substances retained in patients with CRF interfere with bone marrow function. (1) A number of patients with CRF continue to remain anemic despite the presence of elevated serum Ep levels on bioassay⁷⁹ or RIA,^{52, 55} suggesting that the marrow has decreased sensitivity to circulating Ep in these patients. Since some of these studies^{52, 79} did not quantify erythropoiesis by other means such as ferrokinetic studies, one cannot determine whether an appropriate erythropoietic response had occurred in these patients. (2) In nondialysis patients with declining renal function, progressive anemia is noted despite no decrease in serum Ep levels,^{49, 51, 52} suggesting that erythropoietic tissues may be less sensitive to Ep in CRF. (3) Some patients placed on hemodialysis manifest improvement in hematocrit in the absence of significant changes in plasma Ep levels, suggesting that an inhibitor was removed by dialysis.⁸⁰ (4) McGonigle et al. have observed a significant correlation between the degree of *in vitro* inhibition of erythropoiesis and the hematocrit level in patients with CRF. (5) Inhibition of erythropoiesis has been detected in the presence of sera from uremic patients in several tissue culture systems employing both human and animal bone marrow cells, and also in fetal mouse liver cultures.⁸² Uremic sera inhibit proliferation of BFU-E and CFU-E,^{62, 83} heme synthesis,⁸⁴ and marrow thymidine incorporation.⁸⁵

Substances that have been implicated as putative inhibitors of erythropoiesis include spermine and parathyroid hormone. Radtke et al. convincingly demonstrated that the polyamine spermine causes *in vitro* inhibition of erythropoiesis; an antibody to spermine removed the inhibitory effect of uremic serum on erythroid progenitor cells.⁸⁶ However, spermine levels have not been found to be elevated in the plasma of patients with end

stage renal disease⁸⁷ and spermine has been shown to inhibit in vitro granulopoiesis in addition to erythropoiesis.⁸⁸ Partially purified parathyroid extract preparations were found to inhibit erythropoiesis in in vitro studies.⁸⁹ However, the inhibitory effect was found to be nonspecific and was not observed when pure parathyroid hormone, or a synthetic peptide of partial sequence, was tested in culture.⁹⁰ Moreover, parathyroid hormone levels have not been shown to correlate with the severity of anemia in a large group of patients with end stage renal disease.⁹¹

The identity or specificity of the uremic inhibitors of erythropoiesis has not been established. No inhibitor of erythropoiesis identified to date has demonstrated specificity or a significant differential effect on erythropoiesis. Delwiche et al. studied the effect of normal and uremic human serum on in vitro growth of granulocyte—macrophage colonies, erythroid colonies and megakaryocytic colonies in mouse marrow cell cultures.⁹² Their study showed that the uremic sera contained dialyzable inhibitors of in vitro hematopoiesis that increased with the severity of renal dysfunction. However, the inhibitory substances present in uremic sera were not specific for erythropoietic cells only; in vitro granulopoiesis and megakaryopoiesis were also suppressed.⁹² McGonigle et al., on the other hand, found suppression of erythropoiesis but not of granulopoiesis in the presence of sera from patients with renal insufficiency.⁵²

It is possible that the uremic milieu may nonspecifically impair cell proliferation in several tissues. Interference with cell proliferation has been shown in the granulocytes,⁹² intestinal epithelium,⁹³ granulation tissue,⁹⁴ lymphocytes,⁹⁵ as well as male germinal epithelium.⁹⁶

Factors That Aggravate the Decreased Erythropoiesis in Renal Failure

Factors that contribute to the anemia of CRF include deficiency of folic acid,⁹⁷ iron,⁹⁸ and histidine,⁹⁹ as well as protein malnutrition.¹⁰⁰ Iron deficiency may be seen in nontransfused hemodialysis patients because small repetitive blood losses in the dialyser may exceed the absorption of iron from the diet. Iron absorption from the gastrointestinal tract has been found to be normal in patients with CRF.⁹⁸ However, concurrent administration of antacids (used as phosphate-binders) with oral iron effectively chelate iron and interfere with its absorption.¹⁰¹

Subnormal serum folic acid levels are seen in about 10% of uremic patients.⁹⁷ This may be related to increased folate binding proteins in their plasma.¹⁰² Folic acid is a dialysable substance but the amount lost can be easily compensated by intake of a diet adequate in folic acid. Folic acid supplement of 1 mg/day is sufficient to prevent or correct its deficiency.

Osteitis fibrosa, a complication of hyperparathyroidism, may reduce the erythroid marrow space and therefore may reduce erythropoiesis.¹⁰³ The extent of improvement in anemia following parathyroidectomy may be inversely related to the degree of marrow fibrosis.¹⁰⁴

Aluminum toxicity results in microcytic red cells and accentuates the anemia.^{105, 106} Aluminum probably interferes with the incorporation of iron into the erythroid cell for heme synthesis. The microcytosis reverses within 1 to 2 months of treatment of aluminum overload with the chelating agent desferrioxamine, generally before any improvement in hematocrit.

Erythropoietin Deficiency in Chronic Renal Failure

The most important cause of the anemia of CRF is decreased Ep production from the diseased kidneys. When *in vivo* bioassays utilizing radioactive iron incorporation in polycythemic mice were used to measure Ep concentration, serum Ep levels in uremic patients were either undetectable or well below those of comparably anemic patients who have normal renal function.^{8, 107} Although the *in vivo* bioassay is capable of measuring increased circulating levels of Ep, it is not sensitive enough for the measurement of normal or subnormal levels of plasma Ep. Caro et al. concentrated the plasma approximately 200-fold from normal subjects and patients with CRF and then assayed the plasma concentration of Ep using the same polycythemic mouse bioassay.¹⁰⁸ Most patients with CRF had values similar to normal subjects; approximately 40% had elevated levels of bioactive Ep. However, all patients with renal failure had plasma Ep values well below the plasma Ep levels of patients with comparable degree of anemia but without renal failure.¹⁰⁸ Radtke et al. used an *in vitro* bioassay and found serum Ep levels to be higher in all patients with CRF as compared to normal controls.⁴⁹

With the purification of human Ep, valid RIAs for Ep were developed⁴⁴ that measure serum Ep levels as low as 0.4 mU/ml as compared to the sensitivity of the *in vivo* bioassay in unextracted plasma of about 50 mU/ml. The results of serum Ep measurement by RIA have served to clarify whether the relatively low serum Ep levels observed in uremic subjects by bioassays were real or reflected the presence of inhibitors of erythropoiesis in the uremic plasma. The RIA of Ep has shown a high degree of correlation with the polycythemic mouse bioassay.¹⁰⁹ The RIA is not as subject to the influence of enhancing or inhibitory factors in plasma as the *in vivo* or *in vitro* bioassays, even though co-incubation of human urinary Ep with uremic sera was shown to slightly reduce its immunoreactivity in the RIA and biologic activity in an *in vitro* assay.¹¹⁰ Using the RIA, mean values for serum Ep were 18.8 mU/ml for normal females, 17.2 mU/ml for normal males, 97 mU/ml for iron deficiency anemia patients and greater than 1000 mU/ml for patients with anemia secondary to bone marrow failure.¹⁰⁹

We measured serum Ep levels by RIA in 36 hemodialysis patients.⁵⁵ There was a wide scatter in serum Ep levels; although some patients had elevated serum EP, the level was inappropriately low for the degree of anemia in all of the patients. Using RIA, other investigators have also found serum Ep levels to be low relative to the degree of anemia in pa-

tients treated with hemodialysis.⁸¹ The usual correlation between the degree of anemia and elevated levels of serum Ep, as would be expected if renal function were normal,¹⁰⁹ is not seen in patients with CRF.^{55, 81}

Relationship of Serum Erythropoietin Levels and Hematocrit to the Level of Renal Excretory Function

We evaluated the relationship of serum Ep levels measured by RIA to GFR and hematocrit in 48 patients 4 to 22 years of age with chronic renal disease of varying severity and of varying etiologies⁵¹ to evaluate if serum Ep levels change with progressive renal failure. In these patients with chronic renal disease, significant anemia was noted only when GFR fell below 20 ml/min/1.73m². Serum Ep levels, however, did not change with the decline in GFR despite the development of severe anemia (Fig 2). Serum Ep levels did not correlate with hematocrit (Fig 3) or with GFR. McGonigle et al. reported serum immunoreactive Ep levels in 60 patients with varying degrees of renal insufficiency.⁵² Serum Ep levels in patients with renal insufficiency (34.4 ± 6.7 mU/ml) were slightly higher than in

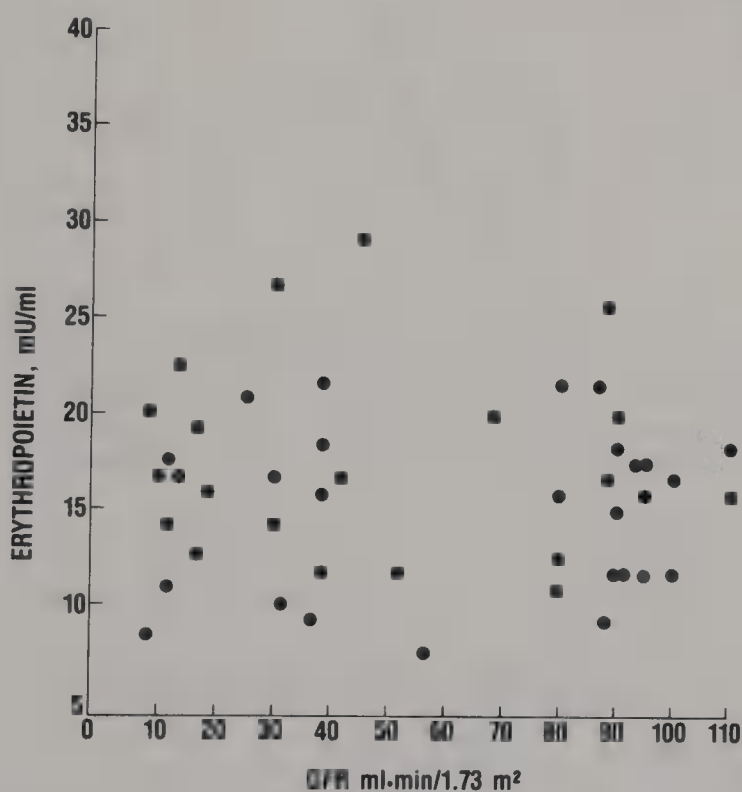
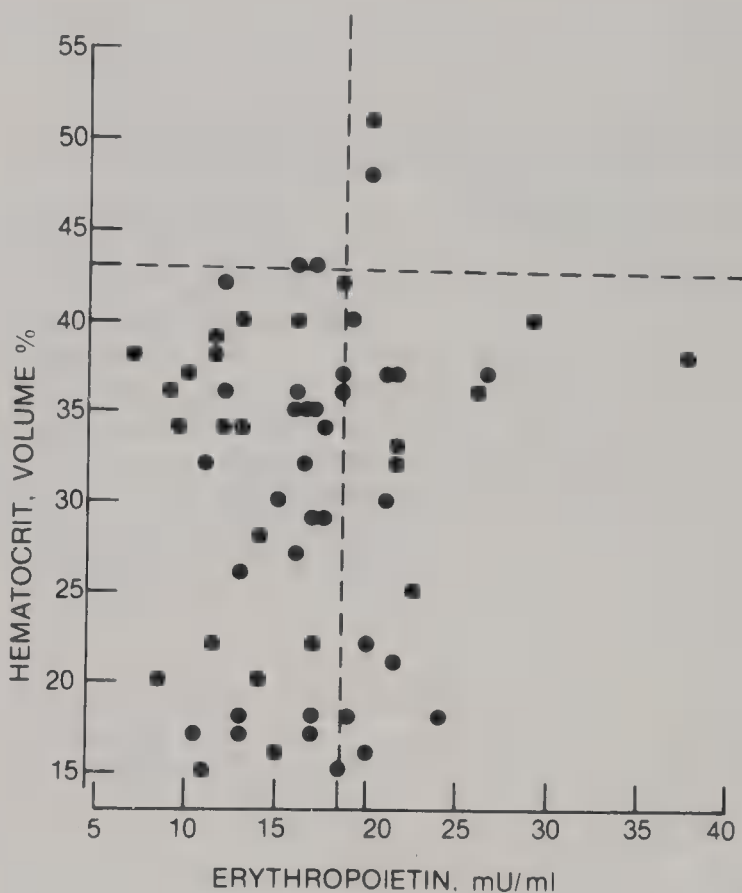


FIG 2.

Relationship of serum Ep levels to the GFR in patients with chronic renal failure (dialysis patients are excluded). (From Chandra M, Clemons GK, McVicar M: Relation of serum erythropoietin levels to renal excretory function: Evidence for lower set point for erythropoietin production in chronic renal failure. Submitted for publication. Used by permission.)

**FIG 3.**

Relationship of hematocrit to serum Ep levels in 48 patients with CRF. The *broken lines* represent the mean values of EP and hematocrit in normal controls. (From Chandra M, Clemons GK, McVicar M: Relation of serum erythropoietin levels to renal excretory function: Evidence for lower set point for erythropoietin production in chronic renal failure. Submitted for publication. Used by permission.)

normal controls (23.1 ± 0.98 mU/ml), but showed no relationship to plasma creatinine, hematocrit, or inhibition of CFU-E formation in fetal mouse liver cultures. Hematocrit and plasma creatinine significantly correlated with the degree of serum inhibition of CFU-E formation.⁵² Radtke et al. measured serum Ep levels in 117 adults with renal failure.⁴⁹ Serum Ep levels did not consistently decrease in parallel with the decrease in GFR. Patients with GFR of 0–9 ml/min had higher mean serum Ep level than patients with GFR of 10–19 ml/min.⁴⁹

■ Hematocrit-Tissue Oxygenation-Erythropoietin Feedback Mechanism Operative in Renal Failure?

We quantitated serum Ep response to acute hypoxia in six children with $\text{GFR} < 20 \text{ ml/min/1.73m}^2$ during spontaneous episodes of acute hypoxic stress.⁵¹ These clinical events included acute pulmonary edema, acute he-

molysis, congestive heart failure, and sepsis with hypotension. Figure 4 shows the serum Ep levels of these six patients during the hypoxic episode (time 0), and either 24–48 hours or 2–4 weeks after such stress. Hematocrit was measured in the same blood sample as Ep. In three patients, serum Ep had been measured during the steady state stable condition prior to the onset of hypoxic stress. In patient 1, serum Ep levels were measured during two episodes of pulmonary edema. Serum Ep values were several times higher during the acute hypoxic stress as compared to serum Ep values during stable state prior to or after such stress (see Fig 4). After the amelioration of the hypoxic stress, serum Ep values fell to levels that were inappropriately low for the degree of anemia in all 6 patients.

Our findings in patients are similar to those reported previously in sheep.⁶⁰ Eschbach et al. showed that phlebotomy of uremic sheep resulted

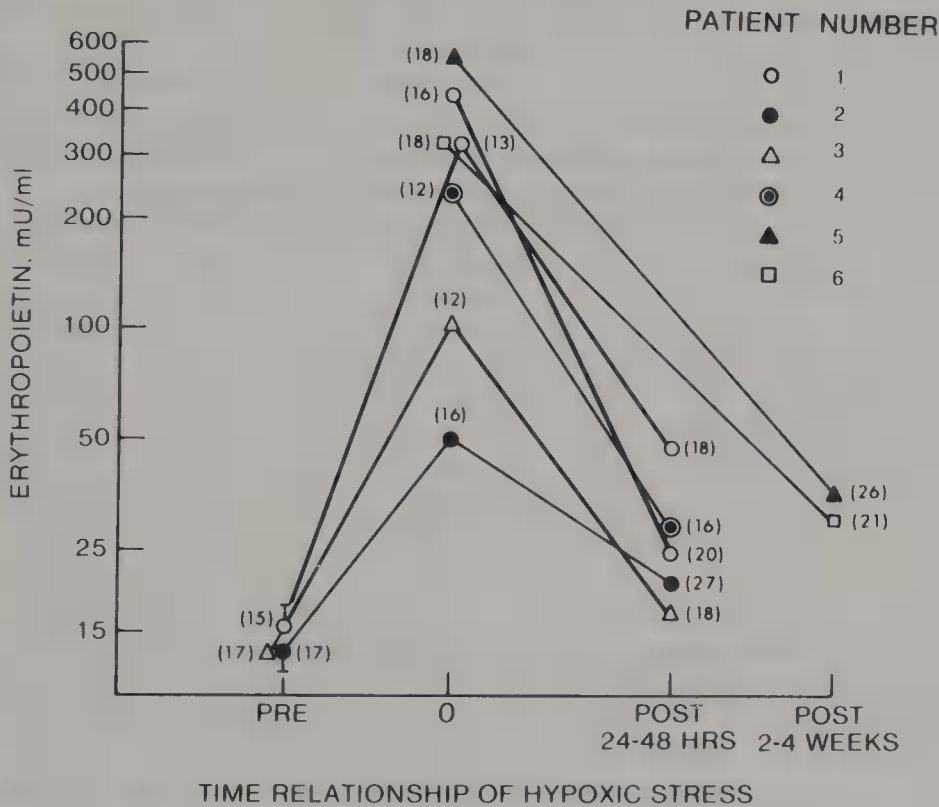


FIG 4. Serum Ep response to acute hypoxic stress in six children with CRF. The numbers in parentheses represent the hematocrit value corresponding to the Ep concentration. In patients 1 and 2 the Ep values (mean \pm SEM), that were obtained prehypoxia on four and five occasions (respectively) are also shown. (From Chandra M, Clemons GK, McVicar M: Relation of serum erythropoietin levels to renal excretory function: Evidence for lower set point for erythropoietin production in chronic renal failure. Submitted for publication. Used by permission.)

in increase in serum Ep and increased erythropoiesis, while administration of blood transfusions to these uremic sheep resulted in a decrease in plasma iron turnover and increase in marrow transit time despite achievement of a final hematocrit that was lower than in normal sheep.⁶⁰ Walle et al. more recently showed a rise in serum Ep in hemodialysis patients in response to spontaneous hemorrhage and suppression in serum Ep levels following blood transfusion.¹¹¹

Why the Erythropoietin Deficiency in Renal Failure?

Though Ep is produced mainly in the kidneys, extrarenal foci of Ep production also exist, the most notable of which is the liver.²⁰ Ep deficiency is, however, seen in renal failure despite availability of the extrarenal site of Ep production. The possible mechanisms for the inappropriately low serum Ep levels in renal failure include: (1) decreased Ep production capacity due to damage of the major Ep producing site, i.e., the kidney; (2) a decrease in the affinity of hemoglobin for oxygen⁵⁷ resulting in increased efficiency of oxygen delivery to tissues and consequently to decreased severity of tissue hypoxia relative to the degree of anemia; (3) unavailability of critical amino acids that are essential for Ep synthesis due to protein malnutrition or abnormal protein metabolism,^{100, 114} and (4) abnormally low set point for Ep production in relation to tissue oxygenation.

The lack of correlation between serum Ep levels and GFR in nondialysis patients with variable degrees of renal insufficiency observed by Chandra et al. and McGonigle et al.^{51, 52} suggests that decreased Ep production is not merely related to the loss of renal parenchyma. The observations that patients with renal failure are able to elevate serum Ep levels in response to acute hypoxia^{51, 111} indicate that lack of availability of critical amino acids for Ep synthesis or lack of Ep production capacity are not the major causative factors for Ep deficiency in renal failure: uremic patients manifest inappropriately low serum Ep levels during stable steady state despite the potential ability to increase Ep production with acute hypoxia.⁵³ These observations suggest that increased Ep production cannot be sustained in uremia or the tissue oxygenation–Ep feedback system operates at a lower set point of tissue oxygen delivery in uremics than in normal individuals. Sufficient observations indicate that a high rate of Ep production can be sustained in some uremic patients. Several hemodialysis patients have been reported who achieved and maintained normal or close to normal hematocrit in response to high Ep production from either diseased native kidneys,^{55, 115} rejected renal allograft,^{116, 117} or regenerating liver.²⁴ In addition, we have reported high serum Ep levels in CAPD patients in stable state.^{53, 118}

The available evidence suggests that the hypoxia–Ep–hematocrit feedback system functions at a lower set point of tissue oxygenation in patients with renal failure than in normal individuals. This may be due to either decreased sensitivity of the renal hypoxia sensor from renal parenchymal damage or the uremic biochemical milieu, or because of inherently higher

threshold for stimulating Ep production from extrarenal sites of Ep synthesis.

Influence of the Mode of Dialysis on the Anemia of Renal Failure and Serum Erythropoietin Levels

An improvement in ferrokinetic studies as well as hematocrit has been noted in patients with end stage renal disease after initiation of hemodialysis treatments. This improvement in hematocrit, however, is not associated with elevation in serum Ep levels.⁸⁰ The improvement in anemia may be related to an increase in hemoglobin-oxygen affinity due to removal of retained phosphates, correction of hyperparathyroidism, better nutrition, or to possible removal of inhibitors of erythropoiesis by hemodialysis.

We evaluated the effect of initiation of CAPD on hematocrit in 35 patients with end stage renal disease.⁵³ The hematocrit values obtained monthly for 3 months prior to starting CAPD and the first 12 months on CAPD were used for analysis. Figure 5 illustrates the significant increase in hematocrit that was noted in CAPD patients within one month of starting CAPD ($P < 0.01$). The hematocrit values obtained at 3, 6, 9, and 12 months after starting CAPD were all significantly higher than the pre-CAPD hematocrit ($P < 0.01$). In some patients, hematocrit did not change at all after starting CAPD while some patients increased their hematocrit

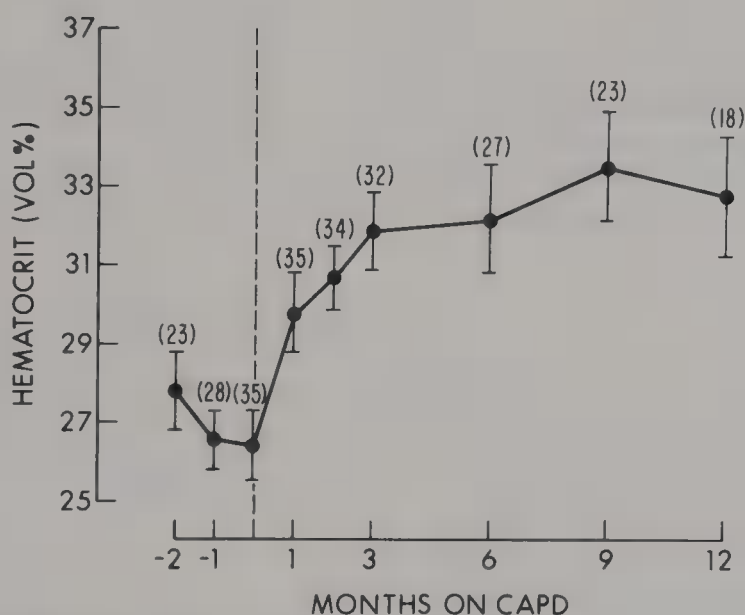


FIG 5.

Relationship between hematocrit and duration of CAPD (mean \pm SEM). Numbers in parentheses represent the number of patients. (From Chandra M, Clemons GK, McVicar M, et al: Serum erythropoietin levels and hematocrit in end stage renal disease: Influence of the mode of dialysis. Submitted for publication. Used by permission.)

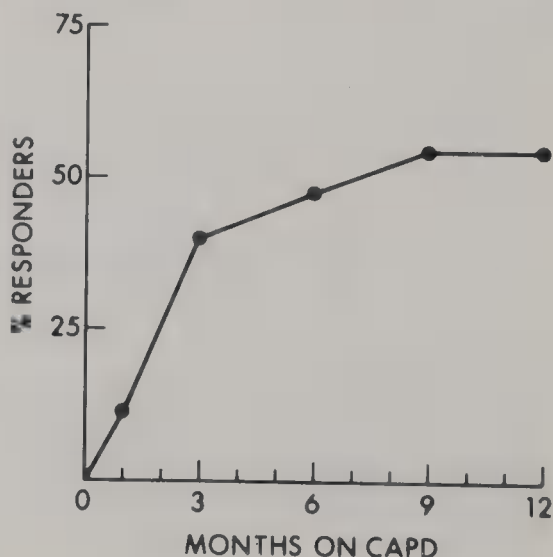
by as much as 17% within 2 months. Three patients manifested a rise in hematocrit to values > 50 volume % at 3 weeks, 6 weeks, and 12 months of starting CAPD. Figure 6 illustrates the percentage of CAPD patients who "responded" with increase in hematocrit by at least 10 volume percent over the pre-CAPD hematocrit or to normal hematocrit ($> 38\%$ in females and $< 42\%$ in males). This response was evident within 1 month of starting CAPD in 11%, within 3 months in 40%, and within 9 months in all 54% of patients who did so. A significant improvement in hematocrit in relation to start of CAPD treatment has also been reported by Depaepe et al.⁵⁴ The increase in hematocrit is predominantly due to an increase in red cell mass and to a lesser extent due to a reduction in the excess plasma volume.⁵⁴ Red cell survival is not found to be significantly altered with CAPD treatment.⁶⁸

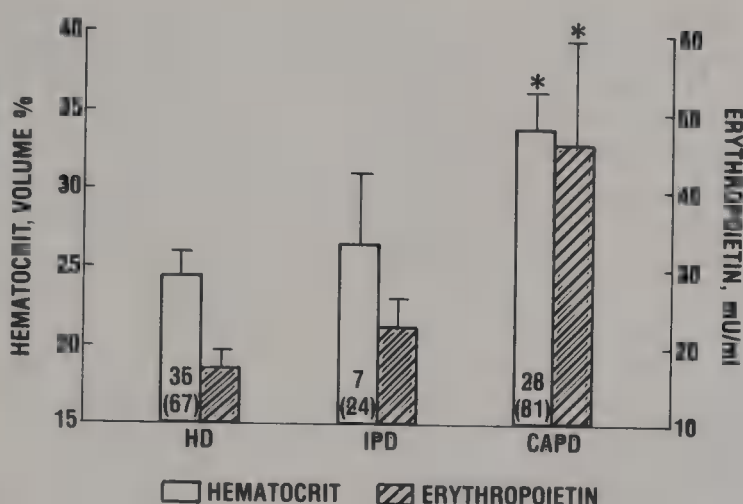
We measured serum Ep levels by RIA in 70 patients on different dialysis treatments to evaluate the influence of the mode of dialysis on the serum Ep levels relative to the severity of anemia.⁵³ Thirty-five patients were on hemodialysis, 7 were on intermittent peritoneal dialysis, and 28 were on CAPD. A total of 172 serum Ep measurements were performed since 42 of the 70 patients had from 2 to 8 blood samples drawn for Ep. Figure 7 illustrates the serum Ep levels and hematocrit in these three groups of dialysis patients. Compared to hemodialysis patients, CAPD patients had higher serum Ep levels and hematocrit ($P < 0.05$). The Ep and hematocrit values of intermittent peritoneal dialysis patients were intermediate between those of hemodialysis and CAPD groups. Our findings suggest that CAPD provides a biochemical milieu more conducive for Ep production than hemodialysis treatment and that the improvement in anemia in CAPD patients may be related to a greater availability of Ep.

Serum Ep levels were found to be higher in six CAPD patients when measured in the first 4 weeks of initiation of CAPD (144 ± 35 mU/ml)

FIG 6.

Relationship between incidence of hematocrit response and duration on CAPD. (From Chandra M, Clemons GK, McVicar M, et al: Serum erythropoietin levels and hematocrit in end stage renal disease: Influence of the mode of dialysis. Submitted for publication. Used by permission.)

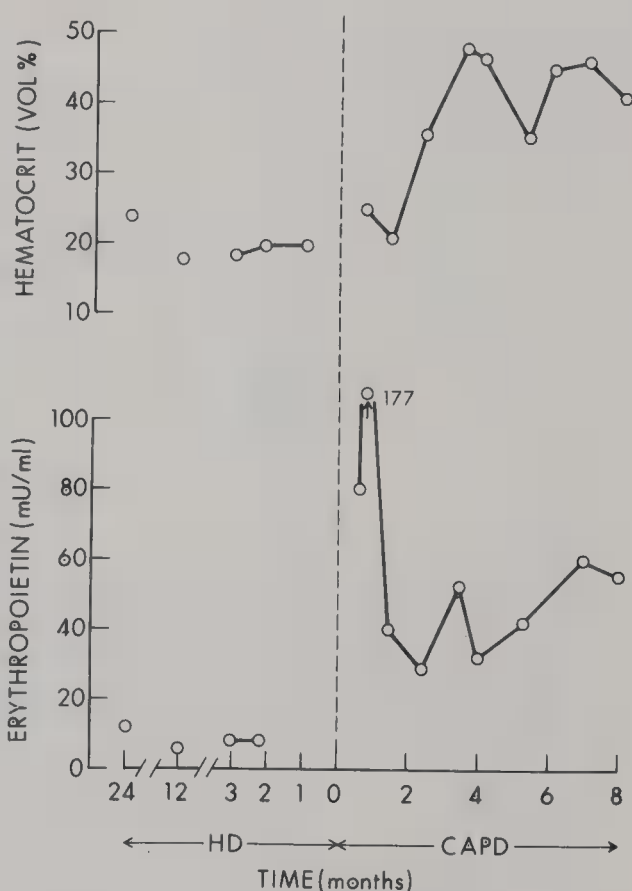


**FIG 7.**

Hematocrit and serum Ep levels (mean \pm SEM) in patients on different modes of dialysis. The numbers in parentheses represent the number of Ep determinations and the upper numbers in the bars represent the numbers of patients. * $P < 0.05$ for CAPD vs. HD group. (From Chandra M, Clemons GK, McVicar M, et al: Serum erythropoietin levels and hematocrit in end stage renal disease: Influence of the mode of dialysis. Submitted for publication. Used by permission.)

(mean \pm SEM) as compared to values obtained in 24 patients later in the course of CAPD treatment (39 ± 6.4 mU/ml).⁵³ A significant elevation in serum Ep levels after starting CAPD was noted in two patients who had serum Ep levels measured while on a different mode of dialysis earlier. We reported¹¹⁸ a 21-year-old female with sickle thalassemia who manifested severe anemia in association with low serum Ep levels of 8.0 ± 1.2 mU/ml while on hemodialysis for 5 years (Fig 8). Serum Ep levels increased to 80 and 177 mU/ml respectively 2 and 3 weeks after starting CAPD. The elevation in serum Ep levels was followed by a rise in reticulocyte count from 3.9% to 22% and in hematocrit from 19% to 48%. Every serum Ep determination obtained during CAPD treatment was higher than that obtained during hemodialysis treatment. The other patient was switched from intermittent peritoneal dialysis to CAPD treatment. His serum Ep levels ranged from 15–26 mU/ml while on IPD for 3 months. Serum Ep values increased to 212 mU/ml within 3 weeks of starting CAPD. This was followed by a rise in hematocrit from 30% to 35% six weeks after starting CAPD.⁵³

Increased serum Ep levels in CAPD as compared to hemodialysis patients are most likely related to increased production, since Ep catabolism or clearance is not significantly influenced by the mode of dialysis. Liver, reticuloendothelial system, and erythropoietic tissues are the major sites of Ep catabolism.¹¹⁹ Increased Ep production in CAPD compared to hemodialysis patients may be related to achievement of better clearance of uremic toxins¹²⁰ that may inhibit Ep production by blunting the sensitivity

**FIG 8.**

Sequential serum Ep concentration and hematocrit in a patient who switched from hemodialysis to CAPD treatments. (From Chandra M, McVicar M, Clemons GK, et al: Role of erythropoietin in the reversal of anemia of renal failure with continuous ambulatory peritoneal dialysis: A case report. *Nephron* 1987; 46:312-315. Used by permission.)

of the hypoxia sensor mechanism. Alternatively the improved state of protein metabolism associated with CAPD¹²¹ or Ep production by dialysate-stimulated peritoneal macrophages may contribute to higher Ep levels.

Possible Role of Peritoneal Macrophages in Erythropoietin Production in CAPD Patients

We observed serum Ep levels to be higher in the first 6 weeks of CAPD treatment than later in the course of CAPD treatment.⁵³ The significant rise in hematocrit noted after starting CAPD treatment is often not sustained,⁵⁴ suggesting that factors other than improved biochemical milieu contribute to increased Ep production in CAPD patients. Since peritoneal macrophages have the potential to produce Ep,²⁸ we evaluated the possibility that the repeated infusion of hypertonic and relatively acidic dialysate in the peritoneal cavity of CAPD patients may stimulate these cells to produce Ep.

Ep concentration was measured by RIA in the dialysis effluent of 13 CAPD patients after an overnight 9-12 hour exchange. The Ep concentration in the dialysate was 2.99 ± 0.24 mU/ml with simultaneous serum Ep concentration of 26.6 ± 4.2 mU/ml.⁵³ The calculated Ep excretion rate in

the dialysate was 560 ± 36 mU/hour of CAPD treatment. Since the volume of distribution of Ep is reported to be equal to the plasma volume,¹¹⁹ the total Ep body pool in these 13 adult CAPD patients calculated to be 70,224 mU at any given time based on their mean hematocrit of 34%, mean serum Ep concentration of 26.6 mU/ml, estimated blood volume of 4 L, and plasma volume of 2.64 L. We calculated the mean Ep excretion rate to be 13,440 mU/24 hours that would represent about 20% of the Ep pool of the body. Since Ep is a relatively large molecule (molecular weight 34,400 daltons), its clearance from the blood in the peritoneal dialysate is expected to be low. The large excretion rate of Ep in the dialysate suggests that Ep may be produced in the peritoneal cavity by peritoneal macrophages. Wideroe et al. showed an increase in the erythropoiesis-stimulating activity in the peritoneal dialysis effluent of CAPD patients as early as 2 days after starting CAPD.¹²² We hypothesize that Ep produced by the peritoneal macrophages may get into the blood stream to a certain extent and contribute to the elevation of serum Ep levels in CAPD patients. In patients treated with intermittent peritoneal dialysis, however, because of the short dwell time and frequent peritoneal lavages, the Ep produced in the peritoneum may not enter the blood stream in significant quantities.

Fluctuations in Serum Erythropoietin and Hematocrit Levels in Dialysis Patients

We noted significant fluctuations in hematocrit and serum Ep values in 42 dialysis patients, when 2 to 8 blood samples were obtained at different time intervals.⁵³ The mean coefficient of variation for serum Ep values was 56.9% for hemodialysis patients, 42.9% for patients on intermittent peritoneal dialysis, and 108% for CAPD patients (Table 3). The fluctuation in Ep values was not due to methodologic problems, because in a normal human serum pool, the intraassay and interassay coefficient of variation for Ep values were 8.4% and 9.7% respectively. Our findings suggest that a single determination of Ep in dialysis patients may not accurately reflect the true erythropoietic status of the patient.

Multiple factors may influence Ep production and its serum concentration in dialysis patients. These factors include (1) plasma volume contraction; (2) changes in hemoglobin-oxygen affinity due to fluctuations in acid base status and serum phosphate concentration¹²³; (3) status of protein malnutrition^{100, 113}; (4) serum levels of renin, angiotensin II and aldosterone^{8, 124, 125}; (5) extent of hemolysis¹²⁶; and (6) renal tissue concentration of calcium and certain prostaglandins.^{38, 127} Since the biochemical environment of the body changes from day to day in dialysis patients, wide fluctuations in serum Ep levels can be expected.

Relationship Between Serum Erythropoietin Levels and Hematocrit in Patients With Chronic Renal Failure

We observed no correlation between serum Ep levels and hematocrit in patients with varying levels of renal function or in hemodialysis pa-

TABLE 3.
Coefficient of Variation in Serum Ep and Hematocrit Values With Multiple
Observations in Dialysis Patients*

Group	No. of Patients	No. of Patients With ≥2 Observations	Total No. of Observations	Range of CV/Patient, %		Mean CV for the Group, %	
				Hct	Serum Ep	Hct	Serum Ep
HD	35	20	67	3.3–33.3	1.1–88.3	24.9	56.9
IPD	7	6	24	3.3–8.3	13.1–26.4	25.4	42.9
CAPD	28	16	81	0–25.2	7.4–119	23.1	108
Total	70	42	172	—	—	—	—

*%CV = $\frac{SD}{Mean} \times 100$; Hct = hematocrit; HD = hemodialysis; IPD = intermittent peritoneal dialysis.

tients.^{53, 57} However, when data from patients treated with different modes of dialysis were combined (see Fig 8), a weak correlation between serum Ep and hematocrit was found ($r = 0.36$, $P < 0.005$). Other investigators have also reported a poor correlation between serum Ep concentration and hematocrit values in patients with renal failure.^{52, 81} This poor correlation between hematocrit and serum Ep may be related to the multifactorial origin of the anemia of renal failure and to several factors that may affect Ep production without influencing hematocrit.^{38, 123-127} Moreover, Ep production at a site not governed by the hematocrit-tissue oxygenation-Ep feedback regulation (e.g., peritoneal macrophages) may contribute to the lack of correlation between hematocrit and Ep.

Is the Bone Marrow Responsive to Endogenous Erythropoietin in Patients With Chronic Renal Failure?

Several studies suggest that Ep is capable of stimulating erythropoiesis despite the uremic milieu. (1) Ep levels are lower in anephric patients than in nephric uremic patients.¹⁰⁷ Bilateral nephrectomies in a stable dialysis patient result in a decrease in erythropoiesis and a fall in hematocrit suggesting that the removal of the source of Ep resulted in further impairment in the low rate of erythropoiesis.¹²⁸ (2) Patients with hemolytic uremic syndrome often manifest a high rate of erythropoiesis as evidenced by increased reticulocyte count despite the presence of renal failure and the need for acute dialysis treatment, and serum Ep levels have been found to be elevated by bioassay in these patients.⁶⁵ (3) Hemodialysis patients with polycystic kidney disease manifest higher erythropoiesis and higher serum Ep levels than patients with renal failure from other renal diseases.⁵⁵ (4) CAPD patients manifest higher hematocrit in association with higher serum Ep levels than do hemodialysis patients.^{53, 118} (5) Several hemodialysis patients have improved or normalized their hematocrit in association with increased serum Ep levels.^{24, 115-118}

Results of Response to Exogenous Erythropoietin Administration in Chronic Renal Failure

Several investigators examined the response of uremic animals to Ep administration. In 1958, Naets showed that erythropoiesis was restored to near normal in eight nephric dogs by infusion of large amounts of Ep.¹²⁹ In two subsequent studies, the anemia of chronically uremic rats was corrected as well as that in nonuremic rats by daily administration of Ep.^{130, 131} Eschbach et al. infused Ep-rich sheep plasma into subtotally nephrectomized uremic sheep, some of whom were maintained on hemodialysis.¹³² Identical erythropoietic responses were elicited by the same doses of Ep-rich plasma in both normal and uremic sheep. In other studies, however, decreased responsiveness of the uremic subjects to exogenous Ep was observed.^{133, 134}

Ep replacement therapy had not been possible in the past because of difficulties involved in purifying large amounts of natural material. A break-

through in the research aiming toward Ep replacement treatment came in 1986 with the availability of large quantities of pure human Ep derived from recombinant DNA. Two clinical trials of recombinant human Ep performed by Eschbach et al. in the USA¹³⁵ and Winearls et al. in UK¹³⁶ have fully confirmed our expectations¹³⁷ that Ep administration can totally correct the anemia of renal failure. Ep was given to hemodialysis patients by intravenous injection three times a week. The response to Ep was found to be uniform, dose-dependent, and predictable at doses above 50 units/kg/dose.¹³⁵ These trials showed that the putative uremic inhibitors of erythropoiesis do not preclude an erythropoietic response. So far, no serious side effects from Ep administration have been reported, although increased incidence of hypertension and thrombosis of the vascular access site has been noted. Hypertension may be the result of increase in blood viscosity due to a rapid increase in the red blood cell mass as well as abolition of hypoxic peripheral vasodilation. A state of functional iron deficiency was noted in some patients secondary to inability of the reticuloendothelial system to release iron to transferrin in response to the high utilization rate of iron in hemoglobin synthesis.^{135, 136}

Summary

In summary, the anemia of CRF results from several interactive processes, chief among these inadequate Ep production relative to the degree of anemia. The anemia of renal failure can be regarded as an endocrine deficiency state, which is corrected by the specific replacement therapy.

The advances of molecular biology have provided a biosynthetic Ep, a potent tool for correction and prevention of the anemia of renal failure. However, new problems often arise as new treatments become available. The rapid improvement in hematocrit and the resultant lowering in plasma volume may affect dialysis clearances in hemodialysis patients. Since the well-being and appetite of the patients improves as the hematocrit rises,¹³⁵ newer methods to increase the weekly dialysis clearances will be needed to prevent the complication of underdialysis in these patients.

References

1. Bright R: Cases and observations, illustrative of renal disease accompanied with the secretion albuminous urine. *Guy's Hosp Rep* 1936; 1:338.
2. Gutman RA, Stead WW, Robinson RR: Physical activity and employment status of patients on maintenance dialysis. *N Engl J Med* 1981; 304:309-313.
3. Jacobson LO, Goldwasser E, Fried W, et al: The role of the kidney in erythropoiesis. *Nature* 1957; 179:633.
4. Erslev AJ: In vitro production of erythropoietin by kidneys perfused with a serum free solution. *Blood* 1974; 44:77.

5. Fisher JW, Langston JW: The influence of hypoxemia induced by cobalt on erythropoietin production in the isolated perfused diseased kidney. *Blood* 1967; 29:114-125.
6. Fried W, Barone-Varelas J, Berman M: Detection of high erythropoietin titres in renal extracts of hypoxic rats. *J Lab Clin Med* 1981; 97:82.
7. Bondurant MC, Koury MJ: Anemia induces accumulation of erythropoietin mRNA in the kidney and liver. *Mol Cell Biol* 1986; 6:2731-2733.
8. Fried W: Erythropoietin and the kidney. *Nephron* 1975; 15:327.
9. Adamson JW, Eschback J, Finch CA: The kidney and erythropoiesis. *Am J Med* 1968; 44:725.
10. Erslev AJ, Caro J, Besarale A: Why the kidney? *Nephron* 1985; 41:213-216.
11. Murphy GP, Mirand EA, Johnson GS, et al: Correlation of renal metabolism with erythropoietin release in hypertensive dogs with renal artery stenosis. *Invest Urol* 1967; 4:372.
12. Schuster SJ, Wilson J, Erslev AJ, et al: Physiologic regulation and tissue localization of renal erythropoietin mRNA. *Blood* 1986; 68(suppl 1): 179a.
13. Jepson J, McGarry EE: Polycythemia and increased erythropoietin production in a patient with hypertrophy of the juxtaglomerular apparatus. *Blood* 1968; 32:370.
14. Demopoulos HB, Highman B, Althland PD, et al: Effects of high altitude on granular juxtaglomerular cells and their possible role in erythropoietin production. *Am J Pathol* 1965; 46:497.
15. Fisher JW, Taylor G, Porteous DD: Localization of erythropoietin in glomeruli of sheep kidney by the fluorescent antibody technique. *Nature* 1965; 205:611.
16. Caro J, Erslev AJ: Biologic and immunologic erythropoietin in extracts from hypoxic whole rat kidneys and in their glomerular and tubular fractions. *J Lab Clin Med* 1984; 103:922.
17. Jelkmann W, Kurtz A, Bauer C: Extraction of erythropoietin from isolated glomeruli of hypoxic rats. *Exp Hematol* 1983; 11:581.
18. Kurtz A, Jelkmann W, Sinowatz F, et al: Renal mesangial cell cultures as a model for study of erythropoietin production. *Proc Natl Acad Sci USA* 1983; 80:4008.
19. Zanjani ED, Poster J, Burlington H, et al: Liver as the primary site of erythropoietin production in the fetus. *J Lab Clin Med* 1977; 89:640.
20. Fried W: The liver as a source of extra renal erythropoietin. *Blood* 1972; 40:661.
21. Gruber DF, Zucali JR, Mirand EA: Identification of erythropoietin producing cells in fetal mouse liver cultures. *Exp Hematol* 1977; 5:392.
22. Paul P, Rothmann SA, McMahan JT, et al: Erythropoietin secretion by isolated rat Kupffer cells. *Exp Hematol* 1984; 12:825.
23. Peschle C, Marone G, Genovese A, et al: Increased erythropoietin production in anephric rats with hyperplasia of the reticuloendothelial system induced by colloidal carbon or zymosan. *Blood* 1986; 47:325.
24. Brown S, Caro J, Erslev AJ, et al: Spontaneous increase in erythropoietin and hematocrit value associated with transient liver enzyme abnormalities in an anephric patient undergoing hemodialysis. *Am J Med* 1980; 68:280-284.
25. Jacobs K, Shoemaker C, Rudersdorf R, et al: Isolation and characterization of genomic and cDNA clones of human erythropoietin. *Nature* 1985; 313:806-810.

26. Lee-Huang S: Cloning and expression of human erythropoietin c-DNA in *Escherichia coli*. *Proc Natl Acad Sci USA* 1984; 81:2708.
27. Fava-De-Moraes F, Zangheri ED, Doine AJ: Immunohistochemical localization of erythropoietin in the rat or mouse submandibular gland. *Histochem J* 1979; 11:97.
28. Rich IN, Anselstetter V, Heit W, et al: Release of erythropoietin from macrophages by treatment with silica. *J Supramol Struct* 1981; 15:169.
29. Spivak JL: The mechanism of action of erythropoietin. *Int J Cell Cloning* 1986; 4:139-166.
30. Till JE, McCulloch EA: A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961; 14:213.
31. Ogawa M, Porter PN, Nakata T: Renewal and commitment to differentiation of hemopoietic stem cells (an interpretive view). *Blood* 1983; 61:823.
32. Iscove NN: The role of erythropoietin in regulation of population size and cell cycling of early and late erythroid precursors in mouse bone marrow. *Cell Tissue Kinet* 1977; 10:323.
33. Embury SH, Garcia JF, Mohandas N, et al: Effects of oxygen inhalation on endogenous erythropoietin kinetics, erythropoiesis, properties of blood cells in sickle-cell anemia. *N Engl J Med* 1984; 311:291-295.
34. Dunn CDR, Smith LN, Leonard JJ, et al: Animal and computer investigations into the murine erythroid response to chronic hypoxia. *Exp Hematol* 1980; 8(suppl):259-279.
35. Stephenson JR, Axelrod AA, McLeod DL: Induction of colonies of hemoglobin synthesizing cells by erythropoietin in vitro. *Proc Natl Acad Sci USA* 1971; 68:1542.
36. Iscove NN, Builbert LJ: Erythropoietin-independence of early erythropoiesis and a two regulator model of proliferative control in the hemopoietic system, in Murphy MJ (ed): *In Vitro Aspects of Erythropoiesis*. New York, Springer-Verlag, 1978, pp 3-7.
37. Nathan DG, Chess L, Hilmann DG, et al: Human erythroid burst-forming unit: T-cell requirement for proliferation in vitro. *J Exp Med* 1978; 147:324.
38. Fisher JW: Prostaglandins and kidney erythropoietin production. *Nephron* 1980; 25:53-56.
39. Mladenovic J, Adamson JW: Adrenergic modulation of erythropoiesis: In vitro studies of colony-forming cells in normal and polycythaemic man. *Br J Haematol* 1984; 56:323-332.
40. Molinari PF: Erythropoietic mechanism of androgens: A critical review and clinical implications. *Haematologica* 1982; 67:442-460.
41. Miyake T, Kung CK-H, Goldwasser E: Purification of human erythropoietin. *J Biol Chem* 1977; 252:5558-5564.
42. Lai PH, Everett R, Wang FF, et al: Structural characterization of human erythropoietin. *J Biol Chem* 1986; 261:3116-3121.
43. Dordal MS, Wang FF, Goldwasser E: The role of carbohydrate in erythropoietin action. *Endocrinology* 1985; 116:2293.
44. Garcia JF, Sherwood J, Goldwasser E: Radioimmunoassay of erythropoietin. *Blood Cells* 1979; 5:405-419.
45. Erslev A: Erythropoietin coming of age (editorial). *N Engl J Med* 1987; 316:101-103.
46. Lin FK, Suggs S, Linc H: Cloning and expression of the human erythropoietin gene. *Proc Natl Acad Sci USA* 1985; 82:7580-7584.

47. Egrie JC, Strickland TW, Lane J, et al: Characterization and biological effects of recombinant human erythropoietin. *Immunobiology* (in press).
48. Egrie JC, Cotes PM, Lane J, et al: Development of valid radioimmunoassays for erythropoietin using recombinant EPO as tracer and immunogen. *Blood* 1985; 66:149a.
49. Radtke HW, Claussner A, Erbes PM, et al: Serum erythropoietin concentration in chronic renal failure: Relationship to degree of anemia and excretory renal function. *Blood* 1979; 54:877-884.
50. Loge JP, Lange RD, Moore CV: Characterization of the anemia associated with chronic renal insufficiency. *Am J Med* 1958; 26:4.
51. Chandra M, Clemons GK, McVicar M: Relation of serum erythropoietin levels to renal excretory function: Evidence for lower set point for erythropoietin production in chronic renal failure. Submitted for publication.
52. McGonigle RJS, Wallin JD, Shaddock RK, et al: Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. *Kidney Int* 1984; 25:437-444.
53. Chandra M, Clemons GK, McVicar M, et al: Serum erythropoietin levels and hematocrit in end stage renal disease: Influence of the mode of dialysis. Submitted for publication.
54. DePaepe MJB, Schelstraete KHG, Ringoir SMG, et al: Influence of continuous ambulatory peritoneal dialysis on the anemia of end stage renal disease. *Kidney Int* 1983; 23:744-748.
55. Chandra M, Miller ME, Garcia JF, et al: Serum immunoreactive erythropoietin levels in patients with polycystic kidney disease as compared with other hemodialysis patients. *Nephron* 1985; 39:26-29.
56. Hilden M, Hilden T: Hemoglobin level and renal function in patients with and without hypertension. *Acta Med Scand* 1968; 183:18.
57. Mitchell TR, Pegrum CD: The oxygen affinity of haemoglobin in chronic renal failure. *Br J Haematol* 1971; 21:263.
58. Neff MS, Kim KE, Persoff M, et al: Hemodynamics of uremic anemia. *Circulation* 1971; 43:876-883.
59. Eschbach JW, Adamson JW, Dennis MB: Physiologic studies in normal and uremic sheep: I. The experimental model. *Kidney Int* 1980; 18:725-731.
60. Eschbach JW, Detter JC, Adamson JW: Physiologic studies in normal and uremic sheep: II. Changes in erythropoiesis and oxygen transport. *Kidney Int* 1980; 18:732-745.
61. Aherne WA: The "burr" red cell and azotemia. *J Clin Pathol* 1957; 10:252.
62. Callen IR, Limarzi LR: Blood and bone marrow studies in renal disease. *Am J Clin Pathol* 1959; 20:3.
63. Moriyama YA, Fisher JW: Effects of erythropoietin on erythroid colony formation in uremic rabbit bone marrow cultures. *Blood* 1975; 45:659.
64. Eschbach JW, Funk D, Adamson J, et al: Erythropoiesis in patients with renal failure undergoing chronic dialysis. *N Engl J Med* 1987; 276:653.
65. Eschbach JW, Adamson JW: Anemia of end stage renal disease. *Kidney Int* 1985; 28:1-5.
66. Welt LG, Sachs JR, McManus TJ: An ion transport defect in erythrocytes from uremic patients. *Trans Assoc Am Physicians* 1964; 77:169.
67. Joske RA, McAlister JM, Prankerd TAJ: Isotope investigations of red cell production and destruction in chronic renal disease. *Clin Sci* 1956; 15:511-522.

68. Hefti JE, Blumberg A, Marti HR: Red cell survival and red cell enzymes in patients on continuous peritoneal dialysis (CAPD). *Clin Nephrol* 1983; 19:232-235.
69. Jacob HS, Amsden T: Acute hemolytic anemia with rigid red cells in hypophosphatemia. *N Engl J Med* 1971; 285:1146.
70. Neiman RS, Bischel MD, Lukes RJ: Hypersplenism in the uremic hemodialysis patient. *Am J Clin Pathol* 1973; 60:502.
71. Bommer J, Ritz E, Waldherr R: Silicon induced splenomegaly: Treatment of pancytopenia by splenectomy in a patient on hemodialysis. *N Engl J Med* 1981; 305:1077.
72. Manzler AD, Schreiner AW: Copper-induced acute hemolytic anemia: A new complication of hemodialysis. *Ann Intern Med* 1970; 73:409-412.
73. Neilan BA, Ehlers SM, Kolpin CF, et al: Prevention of chloramine-induced hemolysis in dialyzed patients. *Clin Nephrol* 1975; 10:105-108.
74. Carlson DJ, Shapiro FL: Methemoglobinemia from well water nitrates: A complication of home dialysis. *Ann Intern Med* 1970; 73:757-759.
75. Orringer EP, Mattern WDL: Formaldehyde-induced hemolysis during chronic hemodialysis. *N Engl J Med* 1976; 294:1416-1420.
76. Berkes SL, Kahn SI, Chazan JA, et al: Prolongs hemolysis from overheated dialysate. *Ann Intern Med* 1975; 83:363-364.
77. Said R, Quintanilla A, Levin N, et al: Acute hemolysis due to profound hypo-osmolality: A complication of hemodialysis. *J Dial* 1977; 1:447-452.
78. Yawata Y, Jacob HS: Abnormal red cell metabolism causing hemolysis in uremia. *Ann Intern Med* 1973; 79:362.
79. Caro J, Brown S, Miller O, et al: Erythropoietin levels in uremic nephric and anephric patients. *J Lab Clin Med* 1979; 93:449-458.
80. Radtke HW, Frei U, Erbes PM, et al: Improving anemia by hemodialysis: Effect on serum erythropoietin. *Kidney Int* 1980; 17:382-387.
81. McGonigle RJS, Hussere F, Wallin JD, et al: Hemodialysis and continuous ambulatory peritoneal dialysis: Effects on erythropoiesis in renal failure. *Kidney Int* 1984; 25:430-436.
82. Fisher JW: Mechanism of the anemia of chronic renal failure (editorial review). *Nephron* 1980; 25:106-111.
83. Ohno Y, Rege AB, Risher JW, et al: Inhibitors of erythroid colony forming cells (CFU-E and BFU-E) in sera of azotemic patients with anemia of renal disease. *J Lab Clin Med* 1978; 92:916-923.
84. Wallner SF, Vautrin RM, Kurnick JE, et al: The effect of serum from patients with chronic renal failure on erythroid colony growth in vitro. *J Lab Clin Med* 1978; 92:370-375.
85. Gutman RA, Huang AT: Inhibition of marrow thymidine incorporation from sera of patients with uremia. *Kidney Int* 1980; 18:715-724.
86. Radtke HW, Rege AB, La Marcke MB, et al: Identification of spermine as an inhibitor of erythropoiesis. *J Clin Invest* 1981; 67:1623-1629.
87. Spragg BP, Bentley DP, Coles GA: Anaemia of chronic renal failure: Polyamines are not raised in uraemic serum. *Nephron* 1984; 38:65-66.
88. Caro J, Erslev AJ: Uremic inhibitors of erythropoiesis. *Semin Nephrol* 1985; 5:128-132.
89. Meytes D, Bogin E, Andrew MA, et al: Effect of parathyroid hormone on erythropoiesis. *J Clin Invest* 1981; 67:1263-1269.
90. Delwiche F, Garrity MJ, Powell JS, et al: High levels of the circulating form

- of parathyroid hormone do not inhibit in vitro erythropoiesis. *J Lab Clin Med* 1983; 102:613-620.
91. McGonigle RJS, Wallin JD, Husserl F, et al: Potential role of parathyroid hormone as an inhibitor of erythropoiesis in the animal of renal failure. *J Lab Clin Med* 1984; 104:1016-1026.
92. Delwiche F, Segal GM, Eschbach JW, et al: Hematopoietic inhibitors in chronic renal failure: Lack of in vitro specificity. *Kidney Int* 1986; 29:641-648.
93. McDermott FT, Dalton MK, Galbraith AJ: The effect of acute renal failure on mitotic duration of mouse ileal epithelium. *Cell Tissue Kinet* 1974; 7:31-36.
94. McDermott FT, Nayman J, DeBoer WGRM: The effect of acute renal failure upon wound healing: Histological and autoradiographic studies in the mouse. *Ann Surg* 1968; 168:142-146.
95. Huber H, Pastner D, Dittrick P, et al: In vitro reactivity of human lymphocytes in uremia: A comparison with the impairment of delayed hypersensitivity. *Clin Exp Immunol* 1969; 5:75-82.
96. Phadke AG, Mac Kinnon KJ, Dossetor JB: Male sterility in uremia: Restoration by renal allograft. *J Can Med Assoc* 1970; 102:607-608.
97. Paine CJ, Hargrove MD, Eichner ER: Folic acid binding protein and folate balance in uremia. *Arch Intern Med* 1976; 136:757.
98. Eschbach JW, Cook JD, Finch CA: Iron absorption in chronic renal disease. *Clin Sci* 1970; 38:191.
99. Giorano C, DeSanto NG, Rinaldi S, et al: Histidine for treatment of uraemic anaemia. *Br Med J* 1973; 4:714.
100. Reissmann KR: Protein metabolism and erythropoiesis. II: Erythropoietin function and erythroid responsiveness in protein-deprived rats. *Blood* 1964; 23:146-152.
101. Rastogi SP, Padilla F, Boyd CM: Effect of aluminum hydroxide on iron absorption (abstract). *Proceedings of the American Society of Nephrology Meeting*. Washington, DC, 1975, p 21.
102. Eichner ER, Paine CJ, Dickson VL, et al: Clinical and laboratory observations on serum folic acid binding protein. *Blood* 1975; 46:599.
103. Weinberg SG, Lubin A, Wiener S, et al: Myelofibrosis and renal osteodystrophy. *Am J Med* 1977; 63:755-764.
104. Zingraff J, Drueke T, Marie P, et al: Anemia and secondary hyperparathyroidism. *Arch Intern Med* 1978; 138:1650-1652.
105. Short ALK, Winney RJ, Robson JS: Reversible microcytic hypochromic anaemia in dialysis patients due to aluminum intoxication. *Proc Eur Dial Transplant Assoc* 1980; 17:226-233.
106. Tauam M, Martinez F, LaCour B, et al: Aluminum-induced, reversible microcytic anemia in chronic renal failure: Clinical and experimental studies. *Clin Nephrol* 1983; 19:295-298.
107. Gurney CW, Jacobson LO, Goldwasser E: The physiologic and clinical significance of erythropoietin. *Ann Intern Med* 1958; 49:363.
108. Caro J, Brown S, Miller O, et al: Erythropoietin levels in uremic nephric and anephric patients. *J Lab Clin Med* 1979; 93:449-458.
109. Garcia JF, Ebbe SN, Hollander L, et al: Radioimmunoassay of erythropoietin: Circulating levels in normal and polycythemic human beings. *J Lab Clin Med* 1982; 99:624-635.
110. McGonigle RJS, Boineau FG, Beckman B, et al: Erythropoietin and inhibitors

- of in vitro erythropoiesis in the development of anemia in children with renal disease. *J Lab Clin Med* 1985; 105:449-458.
111. Walle AJ, Wong Y, Clemons GK, et al: Erythropoietin-hematocrit feedback circuit in the anemia of end-stage renal disease. *Kidney Int* 1987; 31:1205-1209.
112. Anagnostou A, Schade S, Barone J, et al: Effect of protein deprivation on extrarenal erythropoietin production. *Blood* 1978; 51:549-553.
113. Kopple JD: Abnormal aminoacid and protein metabolism in uremia. *Kidney Int* 1978; 14:340-348.
114. Anagnostou A, Schade S, Fried W: Stimulation of erythropoietin secretion by single amino acids. *Proc Soc Exp Biol Med* 1978; 159:139.
115. Shalhoub RJ, Rajan U, Kin VV, et al: Erythrocytosis in patients on long-term hemodialysis. *Ann Intern Med* 1982; 686-690.
116. Chandra M, Garcia JF, Miller ME, et al: Normalization of hematocrit in a uremic patient receiving hemodialysis: Role of erythropoietin. *J Pediatr* 1983; 103:80-83.
117. Zingraff J, Morin D, DiGuilio S, et al: Possible resistant endocrine function of a rejected renal allograft. *Clin Nephrol* 1977; 8:526.
118. Chandra M, McVicar M, Clemon G, et al: Role of erythropoietin in the reversal of anemia of renal failure with continuous ambulatory peritoneal dialysis: A case report. *Nephron* 1987; 46:312-315.
119. Mladenovic J, Eschbach JW, Koup JR, et al: Erythropoietin kinetics in normal and uremic sheep. *J Lab Clin Med* 1985; 105:659-663.
120. Bergstrom J: Serum middle molecules and continuous ambulatory peritoneal dialysis. *Peritoneal Dial Bull* 1982; 2:59-61.
121. Metcalf J, Pederson J, Llach F: CAPD improved intracellular amino acid levels and protein synthesis. *Kidney Int* 1985; 27:182.
122. Wideroe TE, Sanengen T, Halvorsen S: Erythropoietin and uremic toxicity during continuous ambulatory peritoneal dialysis. *Kidney Int* 1983; 24(suppl 16):S208-S217.
123. Torrance JD, Milne FJ, Hurwitz S, et al: Changes in oxygen delivery during hemodialysis. *Clin Nephrol* 1975; 3:54-59.
124. Gould AB, Goodman SA, DeWolf R, et al: Interrelationship of the renin system and erythropoietin in rats. *J Lab Clin Med* 1980; 96:523-534.
125. Fried W, Barone-Varelas J, Morley C: Factors that regulate extrarenal erythropoietin production. *Blood Cells* 1984; 10:287-304.
126. Sanchez-Medal L, Lambardini J: Hemolysis and erythropoiesis. III: The effect of hemolysis and hemolysates on erythropoiesis. *Ann NY Acad Sci* 1968; 149:377-382.
127. Nagakura K, McGonigle RHS, Brookins J, et al: Enhancement of erythropoietin production by calcium entry blockers in response to hypoxia. *Kidney Int* 1986; 29:167A.
128. Kominami N, Laurie EC, Ianhez LE, et al: The effect of total nephrectomy on hematopoiesis in patient undergoing chronic hemodialysis. *J Lab Clin Med* 1971; 78:525.
129. Naets JP: Erythropoiesis in nephrectomized dogs. *Nature* 1958; 181:1134.
130. Anagnostou A, Barone J, Fried W: Effect of erythropoietin on red cell mass of uraemic and non-uraemic rats. *Br J Haematol* 1977; 37:85.
131. Vanstone JC, Max P: Effect of erythropoietin on anemia of peritoneally dialyzed anephric rats. *Kidney Int* 1979; 15:370-375.

132. Eschbach JW, Mladenovic J, Garcia JF, et al: The anemia of chronic renal failure in sheep: Response to erythropoietin rich plasma in vivo. *J Clin Invest* 1984; 74:434-441.
133. Van Dyke DC, Pollycove M, Lawrence JH: Erythropoietin therapy in the renoprival patient, in *Semiannual Report on Biology and Medicine*. Berkeley, Cal., Donner Laboratory, Lawrence Radiation Laboratory, 1967, p 127.
134. Essers U, Muller W, Heintz R: Effect of erythropoietin in normal men and in patients with renal insufficiency. *Proc Eur Dial Transplant Assoc* 1974; 11:398-402.
135. Eschbach JW, Egrie JC, Downing MR, et al: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin: Results of a Phase I and II clinical trial. *N Engl J Med* 1987; 316:73-78.
136. Winearls CA, Oliver DO, Pippard MJ: Effect of human erythropoietin derived from recombinant DNA on the anemia of patients maintained by chronic hemodialysis. *Lancet* 1986; 2:1175-1178.
137. Miller M, Chandra M, Garcia JF: Clinical applications of measurement of serum immunoreactive erythropoietin. *Ann NY Acad Sci* 1986; 459:375-381.

Osteogenesis Imperfecta: Comprehensive Management

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Osteogenesis imperfecta (OI) is a genetic disorder of connective tissue. Its most significant clinical feature is the susceptibility of affected individuals to bone fractures from very mild trauma. In addition to fractures, osseous features of the disorder may include relative macrocephaly, scoliosis, and bowing of the long bones. Although the "brittle bone" aspect of OI is its hallmark feature, OI is a generalized disorder of connective tissue. Affected individuals may have nonosseous features such as blue sclerae, hearing loss, dentinogenesis imperfecta (DI), growth deficiency, cardiopulmonary abnormalities, easy bruising, excessive sweating, and loose or dislocated joints.

Variability is a consistent feature of almost every aspect of OI. Indeed, it may be most productive to consider OI as a group of biochemically and clinically related disorders. In the strict medical genetics sense of the term, i.e., qualitative and quantitative differences in phenotype among individuals carrying the same mutant allele, "variability" in the phenotype of affected individuals is considerable even in the same family. There is also "heterogeneity" among affected individuals in that patients with essentially the same phenotype may have different molecular causes of their disorder. The inheritance of the disorder is variable as well; it may be autosomal dominant or autosomal recessive or may occur as a fresh mutation.

As a result of the collective work of an international group of investigators over the past decade, there has been a surge of new information on the molecular basis of OI. Many individuals with OI have been found to have a defect in their type I collagen genes or protein. The heterogeneity of the mutations that have been reported has prompted an analogy with the multiplicity of hemoglobin mutations in thalassemia.

Some of the new molecular data have directly benefitted patients and their families, especially in terms of improved genetic counselling. Clinical management has also benefitted from the contributions of many specialties in some aspect of OI. Even for severe cases, management has moved beyond passive acceptance of symptoms and minimal treatment to active rehabilitation, and orthopedics supplemented by other subspecialties.

In my view, the "comprehensive" management of a child with OI includes a clinical-radiographic assessment of OI "type," appropriate biochemical studies, genetic counselling for the family, prenatal diagnosis when possible, and early aggressive management of the clinical situation.

Historical Footnote

Since bone is one of the most enduring records of human history, it should not be surprising that specimens resembling OI-afflicted individuals are among ancient anthropologic records. The earliest case with good skeletal documentation is that of an Egyptian mummy¹ dating from about 1000 B.C. The first comprehensive description in the medical literature was in Ekman's doctoral thesis of 1788 on "osteomalacia congenita."² The term osteogenesis imperfecta originated with Vrolik in 1849³; subsequent division of the disorder into "congenita" and "tarda" forms was proposed by Looser in 1906.⁴ Van der Hoeve, in 1918, described the occurrence of fragile bones, blue sclerae, and early deafness as a distinct syndrome.⁵ The terms congenita and tarda have fallen into disuse because they fail to account for the variability of OI and its genetic heterogeneity.⁴

Clinical Classification

In the "congenita" and "tarda" classifications, congenita cases were generally more severe, since, by definition, the diagnosis could be made at birth. This group included the lethal form as well as the more severe surviving cases. There was no accounting for genetic patterns. Tarda cases were those recognized beyond the neonatal period and often overlapped with van der Hoeve syndrome. These cases were generally milder than congenita cases and could anticipate further improvement subsequent to puberty. Emphasis on the time the diagnosis was made failed to account for cases noted at birth in families with a relatively mild form of the disease and the considerable variation in the prognosis of congenita cases. Where was the physician to place families with significant bone disease but lacking in the blue sclerae and hearing loss of the van der Hoeve triad? How were families to be counselled as to the recurrence risk?

The classification now in general use among geneticists was first proposed by Sillence in 1979⁶ and has been subsequently expanded by him and others.⁷⁻¹¹ This clinical, radiographic, and genetic classification divides OI into four types (Table 1). Although a sizable fraction of OI patients do not fit into any of the Sillence OI types as precisely defined, its broad groups have provided a common groundwork for discussion and research. At the Third International Conference on Osteogenesis Imperfecta held in September, 1987, the consensus of the participants was to retain the Sillence classification while research continued to add a more molecular emphasis to the nomenclature.

TABLE 1.
Sillence Classification of Osteogenesis Imperfecta Syndromes

Type	Genetics	Description
I	Autosomal dominant	Mildest form of OI Mild-to-moderate bone fragility without deformity Associated with blue sclerae, early hearing loss, easy bruising May have mild to moderate short stature Type IA: Dentinogenesis Imperfecta Absent Type IB: Dentinogenesis Imperfecta Present
II	Autosomal dominant or recessive	Perinatal lethal Extreme fragility connective tissue, multiple <i>in utero</i> fractures, usually interuterine growth retardation Soft, large cranium Micromelia, long bones crumped and bowed, ribs beaded
III	Autosomal recessive	Progressive deforming phenotype Severe fragility of bones, usually have in utero fractures Severe osteoporosis Relative macrocephaly with triangular facies Fractures heal with deformity and bowing Associated with white sclerae and extreme short stature, scoliosis
IV	Autosomal dominant	Skeletal fragility and osteoporosis more severe than type I Associated with bowing of long bones; light sclerae, \pm moderate short stature, \pm moderate joint hyperextensibility Type IVA: Dentinogenesis Imperfecta Absent Type IVB: Dentinogenesis Imperfecta Present

The severest form of OI in the Sillence classification is called type II (Fig 1) and is, by definition, a lethal condition. Sillence described 16 cases in his original report⁶ and an additional 32 cases in a subsequent subclassification⁸ of the lethal form. Infants are often delivered prematurely or are stillborn, and some are hydropic. Both weight and length are small for gestational age. Affected individuals have extremely poor mineralization and development of the skeleton in utero. The skull is large for

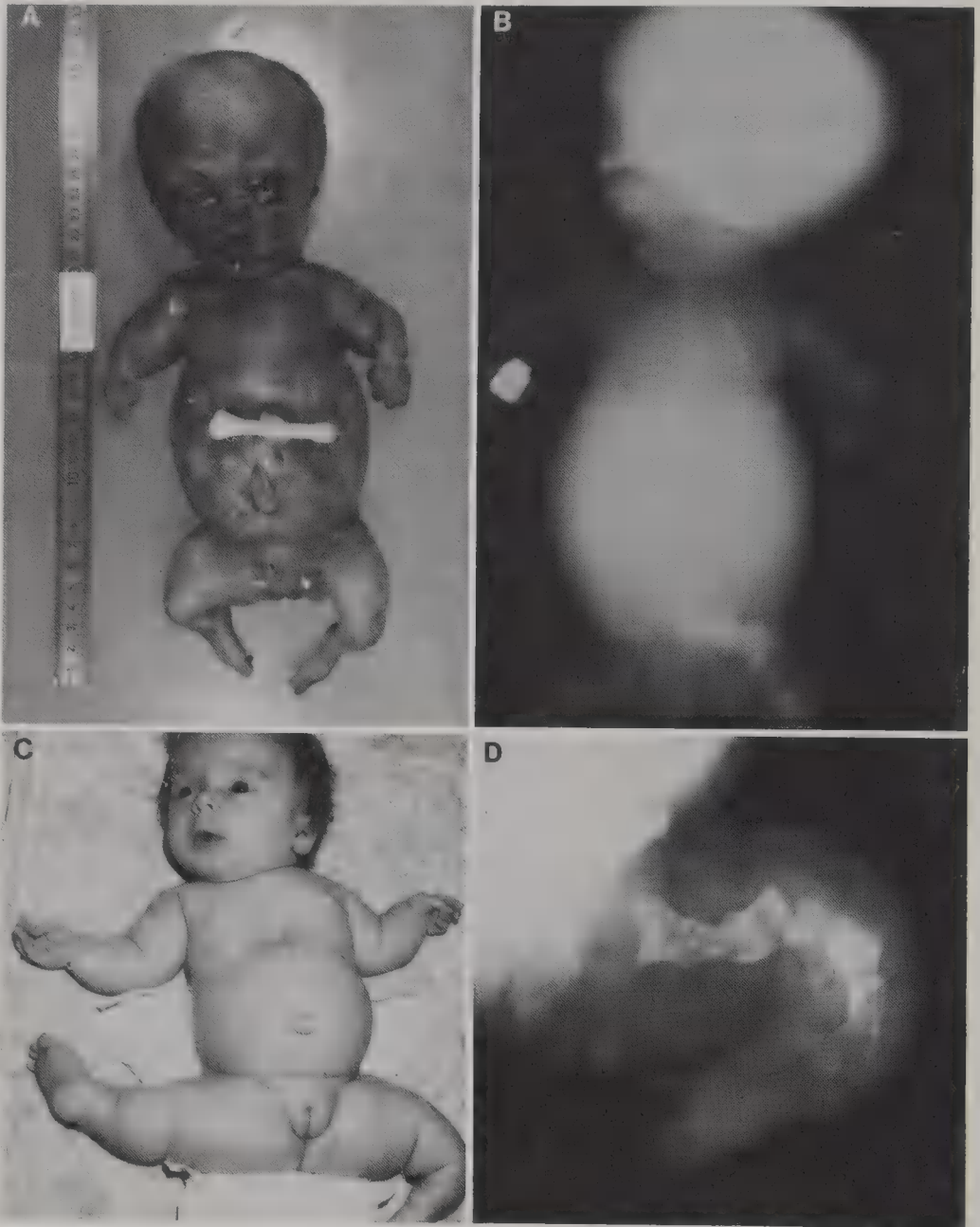


FIG 1.

Infants with type II. **A**, 1.08 kg infant delivered at 32 weeks' gestation who died in the delivery room. Note deformity of limbs and thorax. **B**, radiograph of infant shown in A. Note poorly ossified skull, small thorax, severe hydrops, short and crumpled long bones. **C**, 2-month-old infant who died at 7 months of age with pneumonia. Note severe pectus and deformed limbs. Birth weight was 2.6 kg. **D**, radiograph of leg of infant shown in C. Note long bones are osteoporotic, crumpled and poorly modelled.

body size and poorly mineralized. The so-called caput membranaceum has a memorable soft feeling on palpation and multiple wormian bones on radiographic examination. The triangular facies are rather flat with a small beaked nose. Scleral color is usually a dark blue-gray. The thorax is small and the ribs may feel similar to a rachitic rosary because of callus formation associated with healing in utero fractures. There is striking micromelia and bowing of the extremities; the legs are held abducted at right angles to the body in the "frog-leg" position. Radiographs may be utilized to divide the infants into two groups: one group with continuously beaded ribs and broad, crumpled long bones that are cylindrical in overall appearance and another group with gracile ribs and long bones deformed by fractures.⁸ The type II infants are extremely fragile; vaginal delivery may result in avulsion of body parts or intracranial hemorrhage. Death from pneumonia or respiratory insufficiency secondary to a small thorax is common in those infants surviving delivery. When delivery and neonatal care are optimized for survival, some infants with type II may survive for months to a few years. Pathologic examination of the bones of type II infants has shown normal growth plate cartilage but a marked reduction in collagen in secondary trabeculae and cortical bone.¹² Cortical bone is hypercellular with large osteocytes. Scanning electron microscopy¹³ of a type II case showed larger osteocytic lacunae than in control specimens.

Type II was originally described as a recessive condition because of consanguinity in a number of the families in the original series and the apparent lack of a paternal age effect that might be expected for new dominant mutations. Biochemical data (discussed later) suggest that most type II cases represent new dominant mutations (see also section on genetic counselling) and the Sillence classification has been revised to reflect this information.

Sillence type III (Fig 2) is by definition a severe recessive form of the disease.⁹ This has also been called the "progressive deforming" type. Phenotypically, the presentation at birth overlaps with the milder end of the type II spectrum. Type III disease is compatible with a full life span. However, type III children may die in infancy of respiratory problems; many others will die in childhood with pneumonia, cor pulmonale, or trauma such as skull fractures. For those infants who survive, there is a gradual deformity of the long bones and spine due to fractures and gravitational stress. Osseous fragility is so severe that often the trauma causing the fracture is too mild to be remembered. Facies are characteristically triangular, and the skull is relatively large for trunk size. The blue sclerae of infancy become progressively whiter with age. If DI is present, it is of the transparent greyish variety. Growth deficiency is present in all cases and is generally extreme. Children with severe OI are mentally normal, unless they have suffered significant birth trauma. Surviving patients may be severely handicapped; they traditionally require multiple orthopedic rodding procedures and wheelchairs for mobility. However, aggressive early intervention for rehabilitation and ambulation may change the prognosis for many

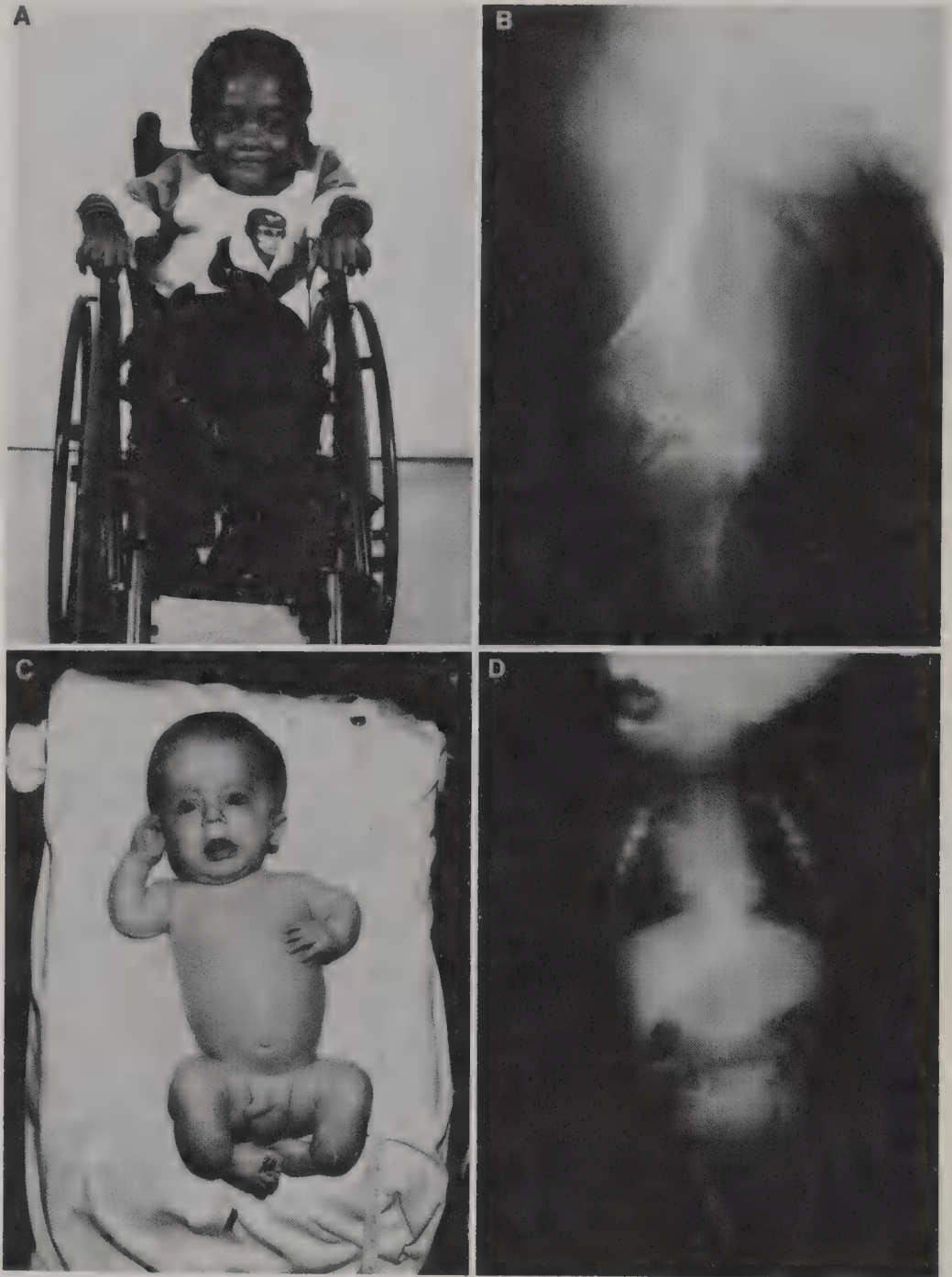


FIG 2.

Children with type III. **A**, 7-year-old boy with triangular facies who is wheelchair bound and has the height of a 3-year-old. **B**, radiograph of femur showing characteristic narrowing of shaft and flaring of metaphyses with "popcorn" appearance of calcified matrix. **C**, 6-week-old infant with severe type III who died age 2 years after a skull fracture. Note large head and severely bowed limbs. **D**, radiograph of infant shown in C. Note beaded ribs and osteoporotic long bones.

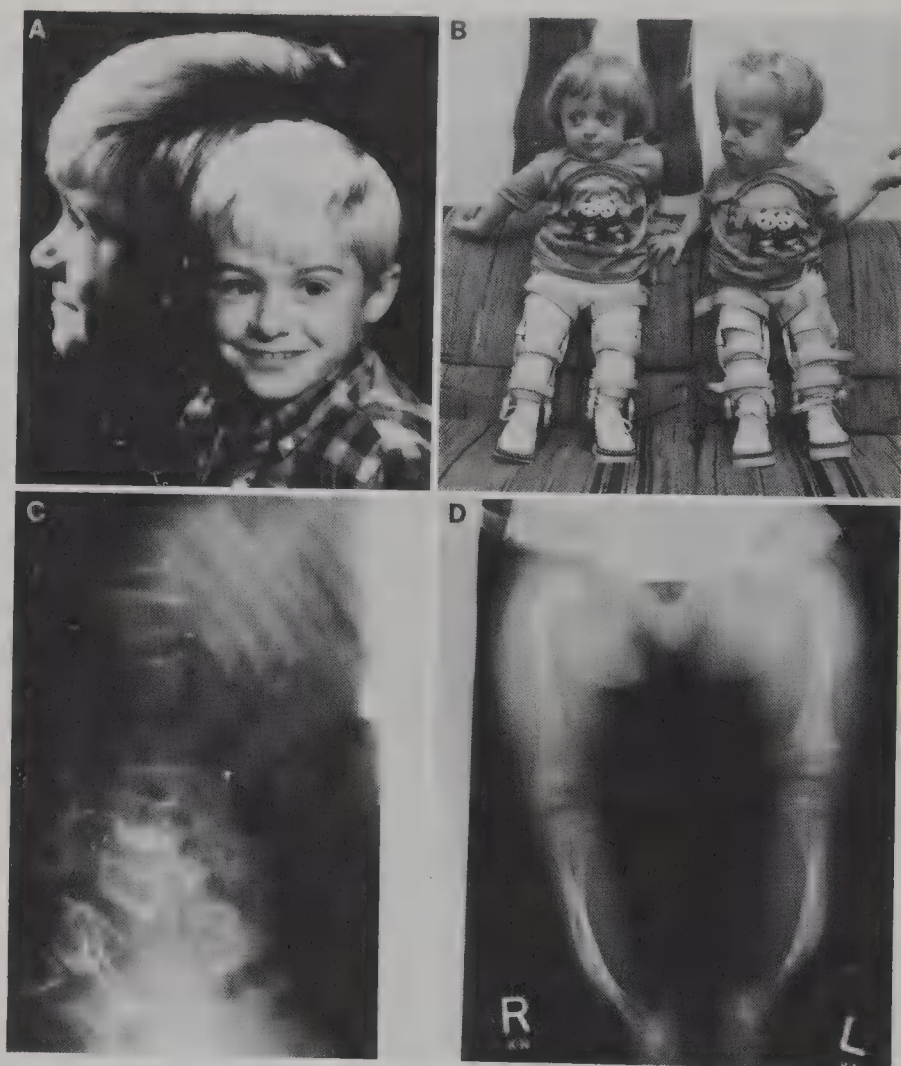
of these children (see section on rehabilitation) at least in terms of self-care potential. Those patients with the most severe bone disease tend not to have associated connective tissue features such as hearing loss, easy bruising, or floppy mitral valves. Some of the parents of these children have been noted to have mild connective tissue abnormalities, such as hearing loss or loose joints, but the significance of this finding is not clear. Radiographically, generalized osteoporosis, wormian bones, "codfish" vertebrae, and multiple deformities are seen. The long bones may be thin and twisted or cylindrical and crumpled. During the childhood years, a cystic "popcorn" appearance of the flared long bone metaphyses gradually develops.

By the strict definition of Sillence, recessive inheritance, as implied (but not proven) by multiple affected siblings born to normal parents or consanguinity of the parents, is essential to the type III diagnosis.⁹ This results in a relatively rare form that does not encompass all of the progressive deforming cases. Sillence reported five such families in one survey⁶ and seven additional families in a later delineation of the phenotype.⁹ Only one type III case has been well defined at the molecular level (discussed later) and its recessive inheritance confirmed.

Most individuals with progressive deforming OI do not have affected relatives and the genetics of these cases is unclear. Since these cases are clinically, radiographically, and prognostically indistinguishable from those that are demonstrably recessive, geneticists in the United States tend to use this category broadly and refer to type III-like phenotypes. Until further molecular studies clarify the inheritance and heterogeneity of the phenotype, it seems to this reviewer useful to keep the "progressive deforming" phenotype in a single group, with designations for cases known to be recessive (R), dominant (very rarely) (D), or unknown (U).

Types I and IV of Sillence (Fig 3) have in common milder bone disease than types II and III and dominant inheritance. The pedigree of an affected child usually reveals multiple family members affected with some features of the disorder. Both types I and IV are divided into A and B subtypes¹¹ depending on whether DI (Fig 4) is present (subtype B) or absent (subtype A). DI tends to breed true in families with this symptom and may be an affected family member's only symptom. A survey of 166 patients from 71 families with type I by Paterson et al.¹⁴ confirmed that DI tends to breed true in pedigrees and demonstrated that individuals from pedigrees with DI have greater fracture rates and more growth impairment.

Many type I families represent van der Hoeve syndrome. In addition to blue sclerae, type I individuals may also have presenile hearing loss (30% to 60%).¹⁵ Significant hearing loss is rarely present before the second decade. It may progress rapidly and many patients require a hearing aid in their third or fourth decade. The hearing loss often represents the most significant long-term handicap of the disorder. Other connective tissue abnormalities may be present in individuals with type I, including mild short stature, mitral valve prolapse, hyperextensibility of joints, easy bruising, and DI.¹⁴ The bone disease itself is the mildest in the OI spectrum. Bones

**FIG 3.**

Children with mild dominant OI. **A**, 10-year-old from family with type I. Child has short stature but otherwise normal appearance. **B**, two children with dominant OI wearing braces for ambulation. The 3-year-old girl (*left*) is sporadic case. Note facial appearance and loose joints. The 4-year-old boy (*right*) has much more severe skeletal manifestations than his mild type I mother. Note macrocephaly. Both children have height of average 1-year-old. **C**, radiograph of spine of child shown in A. Note mild platyspondyly. **D**, radiograph of lower extremities of girl shown in B. Note proximal bowing femurs, thin cortices, and mildly flared metaphyses.

may be mildly osteoporotic but are generally well modeled. The overall "look" of the skeletal system is quite normal. Fractures usually occur after the child begins to walk and are related to moderate trauma. The fractures usually heal without deformity and the fracture rate falls off dramatically after puberty. Women with the disorder appear to have a recurrence of fracture susceptibility after menopause. Expressivity varies among family

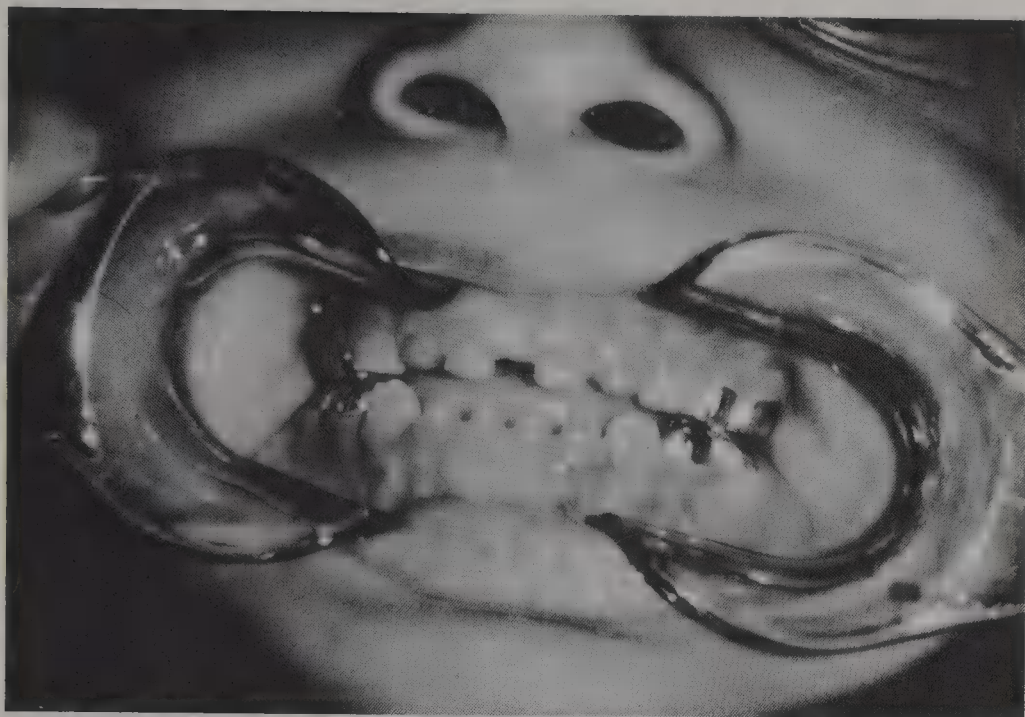


FIG 4.

Teeth of patient with DI. Note that affected teeth are transparent and worn.

members; individuals who have never fractured and have minimal abnormality of their connective tissue on clinical examination have been demonstrated to carry and transmit a defective allele.¹⁶

Type IV¹⁷ is distinct from type I, and cases may be distinguished by their osseous abnormalities (see Fig 3,B,D), which are more severe than those of type I, and their normal sclerae. Some cases of type IV are detected congenitally because of bowing of the long bones or in utero fractures, and other cases are diagnosed when the child begins to ambulate. In childhood, there is usually moderate bowing of long bones, even in children with infrequent fractures. The fracture rate decreases around puberty. The orthopedic aspect of type IV may represent a considerable challenge for ambulation and fracture control; this group may also benefit from aggressive rehabilitation with surgical procedures as needed. Type IV is not associated with presenile hearing loss. Many affected individuals have moderate short stature, and some have dysproportionate short stature. Radiographically, these individuals have moderate osteoporosis and modelling abnormalities of the long bones. Platyspondyly and mild scoliosis may be present.

Many dominant pedigrees do not find an exact niche in the Sillence I and IV classifications. The families most frequently encountered have bone disease resembling type IV but have blue sclerae. The Sillence classification stresses scleral hue as a distinguishing feature between types I and IV.

In the context of the Sillence classification, "blue" refers to a hue that can be easily distinguished by an observer rather than to a subtle tint. It is not yet clear to what extent this emphasis on scleral hue will hold as a discriminating feature. In the United States, many geneticists with connective tissue expertise emphasise the severity of the bone disease and group these families as type IV-like OI. Molecular studies on type IV disease by Wenstrup et al.¹⁸ tend to support the grouping of dominant patients by severity of skeletal manifestation. Similar protein defects are found in patients with type IV-like OI and either light or blue sclerae.

Incidence of OI

The overall frequency for OI identifiable at birth is about 1 in 20,000 to 30,000. The survey of Victoria, Australia, by Sillence⁸ yielded a type II frequency of 1 in 62,000 births and a type III frequency of 1 in 68,000 births; the 4-year survey of Edinburgh birth records showed a frequency of types detectable at birth of 1 in 20,000.¹⁹

Sillence estimated the minimum frequency of type I in Victoria, Australia, as about 1 in 30,000.⁷ This does not include those dominant pedigrees that do not fit the strict type I classification or, of course, pedigrees with very mild manifestations that have not come to medical attention; therefore, this should be considered a minimum estimate.

In the experience of McKusick,²⁰ OI vies with Marfan's syndrome for highest incidence among the connective tissue disorders, although OI appears to be a more heterogeneous syndrome. There is no preferential distribution of OI by gender, race, or ethnic origin.

Molecular Developments in OI

In the last decade, great strides have been made in our understanding of the molecular basis of OI. Many individuals with OI have defects in their type I collagen protein. As is often true for information derived from the study of nature's experiments, this research has added to our understanding of the normal function of type I collagen and the nature of recessive and dominant mutations. The structure and function of the type I collagen genes and protein provide a framework for the presentation of illustrative case studies. Several excellent reviews offering more detail are available.²¹⁻²⁴

A distinction needs to be clearly drawn between the collagen-vascular disorders, which are primarily autoimmune disorders that impact on the connective tissue, and connective tissue disorders, in which the primary defect resides in a component of the connective tissue matrix. In human bone, skin, and tendon, the primary component of the matrix is type I collagen. One of the fibrillar collagens, type I collagen is a heterotrimer

composed of three protein chains that are twisted around each other in a long right-handed helix (Fig 5). Two of the protein chains in the helix, the $\alpha 1(I)$ chains, are identical and are the product of a gene on chromosome 17.²⁵ The other chain is called the $\alpha 2(I)$ chain and is encoded by a gene on chromosome 7.²⁶ The chains are each composed of about 1,000 amino acids with the regular repeating sequence of gly-X-Y, where gly is glycine and X and Y are frequently proline and hydroxyproline, respectively.

Much of the information on the structural-functional relationships in the type I collagen genes came from the work of the de Crombrughe and Boedtker labs on chick fibrillar collagens.^{27, 28} Recently, Ramirez, Myers, and others²⁹⁻³¹ have cloned most of the two human type I collagen genes and demonstrated conservation of structure. The type I collagen genes are composed of about 50 exons (coding regions) scattered over 18 kilobases (for $\alpha 1(I)$) and 38 kilobases (for $\alpha 2(I)$) of chromosomal material. Most of the exons consist of 54 or 108 base pairs. Each exon codes for an even multiple of the repeating gly-X-Y unit beginning with a gly codon and ending with a codon for the Y amino acid. This structure led researchers to postulate that the genes arose by duplication of a functional 54 base pair unit. Initial mRNA transcripts are spliced and processed as are those from other eukaryotic single copy genes; the final coding mRNAs in the cytoplasm range in size from 5.5 to 7.2 kilobases.³¹

The protein chains composing type I collagen are synthesized as procollagen forms, with globular extensions at both the amino and carboxyl ends of the central helical portion (see Fig 5). The three chains composing the helix associate in the proper alignment and composition, as directed by sequences in the carboxyl-terminal extensions. From the carboxyl end, helix formation proceeds toward the amino terminal end (Fig 6,B). Simultaneously, proline and lysine residues are hydroxylated by specific hydroxylases³² and the hydroxylysine positions are glycosylated by sugar transferases.³³ These modifications occur only on chains that are in non-

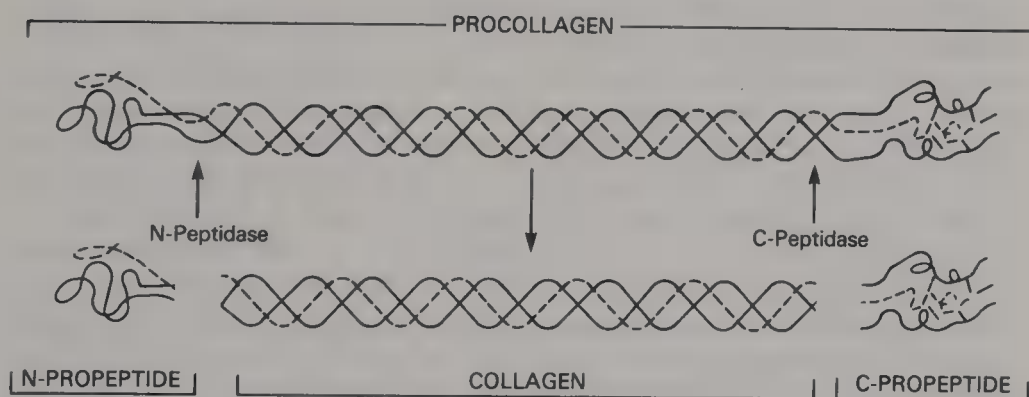
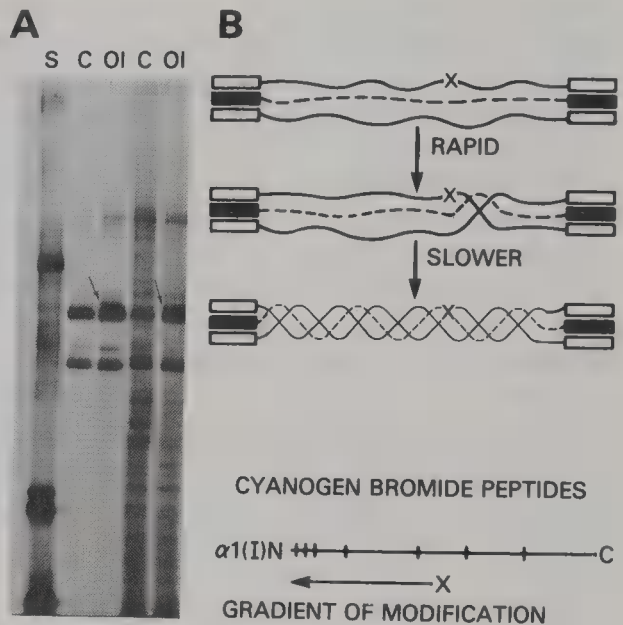


FIG 5.

The procollagen molecule and its processing to the mature form.

FIG 6.

Overmodification of type I collagen characteristically seen in type II. **A**, SDS gel of collagens from perinatal lethal OI case. S = protein standards; C = control. Arrows indicate the overmodified form of the $\alpha 1(I)$ chain present in the OI media (lanes on left) and intracellularly (lanes on right). (Courtesy of Dr. D.K. Grange.) **B**, helix formation from carboxyl to amino ends and gradient of overmodification due to the point mutation.



helical configuration. Both interchain and intrachain disulfide cross-links form in the terminal extensions.

Current evidence indicates that type I collagen is secreted from the cell in its pro form. In the extracellular space, specific N- and C-peptidases (see Fig 5) cleave the terminal extensions.³⁴ The mature helical molecules then self-aggregate into the well-known, quarter-staggered array. Lastly, the fibrils are stabilized by intermolecular cross-links formed by oxidative deamination of lysine and hydroxylysine residues. The final structure owes its functional strength to precise chain alignment, a temperature-stable helix, proper modifications, and a final size that fits the fibrillar array. Mutations that interfere with these structural properties would be predicted to have substantial functional consequences.

The collagen defects associated with Sillence type II have been studied most thoroughly and have been found to be heterogeneous at the molecular level. A small percent of the cases examined are characterized by a deletion of a portion of the gene. For example, the cell line CRL 1262 from a lethal case has been shown to synthesize both normal length and shortened pro $\alpha 1(I)$ chains.³⁵ At the gene level, one allele contains a deletion of 643 bp of coding sequence; the end points of the deletion^{36, 37} are in introns so the coding sequences remain in register and produce a chain shortened by 84 amino acids (residues 328-411). The shortened chains still contain normal association sequences in the carboxyl-propeptide and participate in trimer formation. Other lethal cases of OI have been associated with deletions in one allele of the $\alpha 2(I)$ gene³⁸ (Byers, personal communication) or an insertion in an $\alpha 1(I)$ allele (Byers, personal communication).

Point mutations in type I collagen alleles have also been associated with

perinatal lethal OI. Steinman and co-workers³⁹ reported a case in which a point mutation from glycine to cysteine occurred at position no. 988 of the $\alpha 1(I)$ chain and was detected by the formation of reducible cystein dimers in trimers containing two copies of the mutant chain. Bateman and co-workers reported two point mutations in $\alpha 1(I)$ detected as charge changes, a glycine to arginine change at position 391⁴⁰ and a basic charge change in cyanogen bromide peptide 7.⁴¹ In each of these cases, the type I collagen chains are overmodified from the site of the mutation to the amino terminal end (see Fig 6,B). The mutations are thought to interfere with the rate of helix propagation and to allow time for more modification of the constituent chains than usually occurs.

However, most cases of type II do not involve deletions or easily detectable point mutations. When the type I collagen from the fibroblasts of these patients is examined,⁴¹⁻⁴³ the cells are found to synthesize both normal procollagen chains and abnormal chains that migrate more slowly on gels (see Fig 6,A). The slow form has been shown to be due to overmodification of the collagen; fibroblasts from lethal cases do not synthesize abnormally migrating chains when incubated with an inhibitor of proline hydroxylation. When the cyanogen bromide fragments of the overmodified collagen of these patients were examined, a carboxy to amino terminal gradient of overmodification (see Fig 5,B) was noted, just as for the point mutations described earlier. This observation has prompted the proposal that these cases are also due to point mutations that impede the normal rate of helix propagation.

The study of these mutations has provoked new awareness of the nature of dominant and recessive mutations. In diseases due to defects in crucial metabolic enzymes, the presence of one functional allele provides adequate enzymatically active product for a normal phenotype. The severe phenotype in enzymatic defects occurs when two defective alleles are present; it is recessive in inheritance. Defects in collagen alleles will have different consequences because the molecule is a multimer and because it plays a structural rather than an enzymatic role. For example, the random association of half-normal and half-mutant $\alpha 1(I)$ chains into type I collagen results in three fourths of the total product having at least one mutant chain. This amplification of the consequences of a single mutant allele has been termed protein suicide by Prockop.⁴⁴ Trimers with mutant components form unstable helices and are poorly secreted. The secreted trimers, normal plus abnormal, are inadequate to support the normal structural role of type I collagen; hence, these single allele mutations behave in a dominant, rather than a recessive, fashion.

In all of the cases of type II with rearrangements or overmodification, the parental collagens do not demonstrate a similar abnormality. This suggests that these occur as spontaneous mutations. A number of couples with normal fibroblast collagens have had more than one affected child with overmodified collagen. It is not yet clear whether these cases repre-

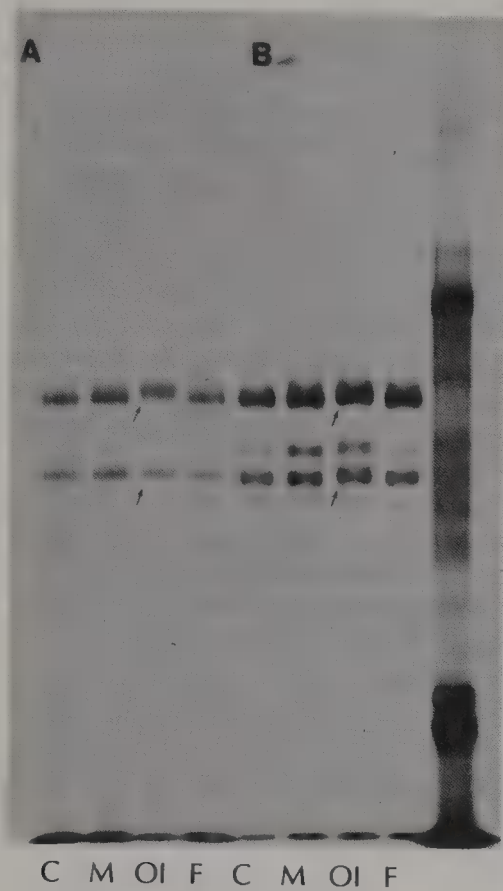
sent compound mutations or parental gonadal mosaicism (see section on genetic counselling).

The molecular abnormalities of type III have been defined in only one special case. Numerous other cases show no abnormality of type I collagen when the protein is examined, even at the cyanogen bromide peptide level. The type III case with an abnormality of type I collagen was the product of a consanguineous mating. Each partner carries the same mutant $\alpha 2(I)$ allele with a four-base pair deletion in the carboxyl-terminal peptide.⁴⁵ This deletion results in a frameshift mutation,⁴⁶ altering the protein sequences in the region of the chain crucial for interchain alignment in the type I helix. The child makes $\alpha 2(I)$ chains intracellularly⁴⁷; however, he cannot incorporate them into type I helix, and the secreted collagen is an $\alpha 1(I)$ trimer.

It is tempting to speculate, based on this case, that there is a less severe clinical outcome (type III) from a mutation that is not secreted from the cell than from a deletion that is secreted. As a clinical-pathologic correlation, this is premature; however, the heterozygous parents are interesting. The carrier phenotype in this case is detectable clinically only as moderate early-onset osteoporosis.

Type IV has been associated with several different types of mutations in the $\alpha 2(I)$ chain. In one case,¹⁸ a deletion of ten amino acids from the middle of the triple helical region causes increased post-translational modification in the amino half of some trimers, lowers their melting temperature, and delays their secretion. In another type IV case,⁴⁸ a deletion near the amino terminus of the $\alpha 2(I)$ chain did not result in overmodified type I collagen or poor secretion; however, it is located near the amino-propeptide, and the trimers into which it incorporates are resistant to cleavage by procollagen N-proteinase. Because the proper functioning of type I collagen requires that the mature molecules fit properly into a quarter-staggered array, one would expect abnormalities in collagen size to cause significant symptoms. Other type IV cases simply show overmodification (Fig 7), but not a detectable deletion. Thus, on an SDS gel, the collagens from these patients are difficult to distinguish from those of type II cases. Mutations in $\alpha 1(I)$ alleles as well as $\alpha 2(I)$ alleles have been detected (Byers, personal communication).

Studies of fibroblast collagen from patients with type I reveal a number of defects. The common feature of these cases is a decreased synthesis of structurally normal type I collagen. A pedigree studied by Rowe et al.⁴⁹ had a 50% to 75% reduction in type I collagen production, with elevated $\alpha 1(III)/\alpha 1(I)$ and $\alpha 1(I)/\alpha 2(I)$ ratios. Barsh et al.⁵⁰ demonstrated an intracellular $\alpha 1(I)/\alpha 2(I)$ ratio of 1 instead of the normal ratio of 2. They suggested that one $\alpha 1(I)$ allele was nonfunctional but could not determine whether this was at the transcriptional or translational level. Two groups^{16, 51} reported cases of type I in which the presence of a cysteine mutation in an $\alpha 1(I)$ chain has been detected. The family reported by de Vries and de Wet has a cysteine residue in the region of the chain containing amino

**FIG 7.**

SDS gel of type I collagen chains of child with type IV (girl shown in Fig 3, B). Note that when the collagen chains of the child (arrows) are compared with controls (C) and her mother (M) and father (F), her chains migrate more slowly. **A**, intracellular collagens; **B**, cellular collagens.

acids 124 to 402. As a result of the mutation, triple helical molecules containing the mutant chain were overmodified, had a lower melting temperature, and were poorly secreted. The identity and location of the amino acid for which the cysteine substitutes are not yet known. The family reported by Nicholls and Pope⁵¹ has a cysteine residue near the carboxyl-terminal end of one $\alpha 1(I)$ chain.

A complementary approach to the molecular study of OI is the use of restriction fragment length polymorphisms (RFLPs). These studies utilize variations in base sequences that are present in the entire population and that can be detected by restriction enzymes. RFLPs are used to trace the inheritance of various alleles of a gene through a dominant pedigree to determine if inheritance of an allele segregates with inheritance of the disease phenotype. Several RFLPs are available for the $\alpha 2(I)$ gene and their use has been widespread⁵²; only recently have two RFLPs become available for $\alpha 1(I)$.⁵³

In summary, dominant OI in many pedigrees has been tightly linked to the $\alpha 1(I)$ or $\alpha 2(I)$ polymorphisms. Mild dominant forms of OI are thus heterogeneous in etiology although there is a strong association of type IV with $\alpha 2(I)$. Unfortunately, RFLPs are useful only for the study of dominant forms of OI with multiple, affected individuals in several generations. The

availability of RFLPs for both genes coding for type I collagen will permit a more formal investigation of whether the dominant OI loci are limited to the type I collagen genes.

The demonstration of abnormalities in the genes and proteins for type I collagen is, strictly speaking, presumptive evidence that those abnormalities are the primary defect in the disorder. For example, overmodification might have been a secondary effect of another mutation. It is formally difficult to prove that point mutations to cysteine are the cause of the disorder and not a second unrelated change. One way to approach this molecular dilemma is to synthetically induce mutations onto a normal genetic background and to observe the resulting phenotype. The MOV-13 mouse studied by Jaenisch and co-workers has provided such a system. In this strain the $\alpha 1(I)$ gene has been inactivated by retrovirus insertion.⁵⁴ MOV-13 heterozygotes contain 50% of the normal amount of type I collagen and may be considered to be a model for type I OI. Homozygosity for this mutation is a lethal condition; embryos die at 12 to 14 days of gestation of blood vessel rupture.⁵⁵ Tissue culture cells from homozygotes synthesize $\alpha 2(I)$ chains but they are rapidly degraded intracellularly because there is no $\alpha 1(I)$ to form a protective trimer. Stacey and co-workers demonstrated that they can "rescue" the $\alpha 2(I)$ chains by vector-mediated transfer of the human pro $\alpha 1(I)$ collagen gene into MOV-13 cells⁵⁶ and that functional mouse-human hybrid type I collagen results.⁵⁷ The ability to transduce a mutant pro $\alpha 1(I)$ gene into the heterozygote background should enable this group to define the functional effects of specific mutations. Preliminary experiments (Jaenisch, personal communication) have shown that the introduction of cysteine and arginine mutations at glycine 859 results in poorly secreted type I collagen with a carboxyl to amino gradient of overmodification in vitro and a dominant lethal phenotype in mice that clinically and biochemically resembles the perinatal lethal OI of humans.

The exciting data from the MOV mouse system provide reassurance that the mutations detected to date in type I collagen are the primary mutations in OI. However, knowledge of the etiology of the disorder has not provided an equal understanding of the pathogenesis of the disease and the relationship of particular mutations to disease severity. For example, both types II and IV are associated with mutations that result in overmodification of the type I collagen, yet they have widely different phenotypes. Even within the type II cases, there is no direct relationship between the severity of the case and the extent of overmodification. The overmodification is likely to be a secondary effect of the mutation and is most useful as a biochemical marker. Likewise, point mutations to cysteine, which cause helix disruption, have been demonstrated in both type II and type I,⁵⁸ quite the opposite ends of the clinical spectrum. One possibility is that the determinant of clinical severity is the chain on which the mutation is located or the relative position on the chain, i.e., mutations in the carboxyl half of the mature helix may be associated with greater clinical severity than those in the amino end. Mutations in a certain region may result in a

particular phenotype because that region is crucial for interactions with other matrix proteins. Variability, even within a family, might result from differences in the noncollagen genetic background as well as from compound mutations for collagen. Continued investigation of the collagen genes in OI patients should add to understanding of this aspect of the pathogenesis.

Furthermore, to understand the pathogenesis of the skeletal aspects of OI, we need to understand the way(s) in which the primary defect alters bone cell metabolism and bone matrix interactions. For example, several investigators^{59, 60} have reported the presence of type III collagen in bone from patients with lethal OI. Specific antisera have localized the type III collagen to the compacta region of the bone. Normal bone contains only type I collagen except for the cells lining the marrow cavity. Also, osteoblasts from a perinatal lethal case with overmodified type I collagen handled the production of PG1 abnormally (Grange, Robey, and Marini, unpublished results), with a several-fold increase of PG1 and about a 60% decrease of PG2. Abnormalities in the intracellular metabolism of a major product of the cell (collagen) may be generally disruptive of matrix production and cause the phenotype of the disease secondarily.

In many cases of OI, especially type III, it has not been possible to find a defect in the patient's collagen. Some of these cases may represent point mutations that are undetectable by current screening methods. In other cases, defects in matrix genes other than collagen may be responsible for the disorder. Evidence suggestive of this possibility comes from the work of Termine, Robey, and Fisher on Australian and Texan cattle that have a brittle bone disorder similar to the human type III.⁶¹ The bone proteins implicated in their studies are osteonectin, a bone glycoprotein with high affinity for both collagen and bone mineral, and bone proteoglycan. The bones of the Texan OI cattle have only 2% of the normal level of osteonectin and decreased bone proteoglycan. The bone cells from these animals do make osteonectin and bone proteoglycan when grown in culture, but they do not become incorporated in the extracellular matrix. Whole bone from the Australian OI cattle has normal amounts of osteonectin and dramatically reduced levels of bone proteoglycan. Both products are made by the Australian bone cells in culture but only reduced amounts are incorporated into matrix. Some human OI patients with apparently normal collagen have reduced amounts of osteonectin in their bones.

Genetic Counselling

Genetic counselling is a complex process in which patients and families are given information as to diagnosis, the prognosis connected with the diagnosis, the recurrence risk, the possibility of prenatal testing, and the availability of support systems.

For the parents of a child with the lethal form of OI, the counselling for

recurrence risk generally conveys good news. As discussed earlier, most cases of perinatal lethal OI appear to be due to new mutations that behave in a dominant fashion. When appropriate collagen studies confirm that the child has a collagen abnormality that is not manifest in the parents, the recurrence risk to the parents is very low. Nonetheless, a small percent of such families (5% to 7%) do experience recurrences of the lethal form. There is currently no way to determine in advance which families are at higher risk for recurrence, although the type II-C subtype has been associated with affected siblings.^{8, 62} In the families with two or more affected offspring, the cause may be parental gonadal mosaicism or a genetic compound. Prenatal monitoring, at least by ultrasound, is clearly warranted.

The parents of a child with progressive deforming OI but no history of OI in the family are very difficult to counsel. The laboratory tests have a low yield in helping the counselor or family. It is our experience that some parents will have "soft" connective tissue abnormalities but no fracture history. The empirical recurrence risk to parents of an affected child is only a few percent but the statistics are distorted since couples will often refrain from having additional children after the birth of a severely affected child. It seems prudent, therefore, to counsel couples that the recurrence risk for them may be from 5% to 25%. The mean paternal age of type III cases was not found to be significantly increased over the general population.⁶³ For those individuals with progressive deforming OI who seek counselling with an unaffected spouse concerning their risk of producing an affected child, the answer is more cautious. Here, it would seem most prudent to explain how the individual might carry a spontaneous mutation that would be passed to offspring in a dominant fashion (50% recurrence risk in each pregnancy). For example, we have seen two families illustrating this pattern (Fig 8). In family A, a severely affected father and his spouse with no ostensible connective tissue disease have two sons who greatly resemble each other in clinical severity but have much milder disease than their father. In family B, one parent has progressive deforming OI and had a sibling who died in infancy with severe OI. The first child of this man and his clinically normal spouse has severe OI. Some paternal relatives are reported to have blue sclerae. Pending further molecular information on such a family, counselling for up to a 50% recurrence risk is warranted.

Counselling for types I and IV would seem to be more straight-forward since the dominant inheritance is clearer. However, even in large pedigrees, counselling is complicated by the variability of the disease and new molecular knowledge that even individuals with the mildest of symptoms can be carriers.¹⁶ For example, we have seen a family in which a woman with DI and her clinically normal spouse have two moderately severe offspring. The disease does usually breed true, that is, an affected individual can expect the severity of symptoms to be within the severity range exhibited by his or her affected family members; however, occasional surprisingly severe cases (progressive deforming phenotype) appear in mild dominant pedigrees (see Fig 3,B). It is not clear whether these children

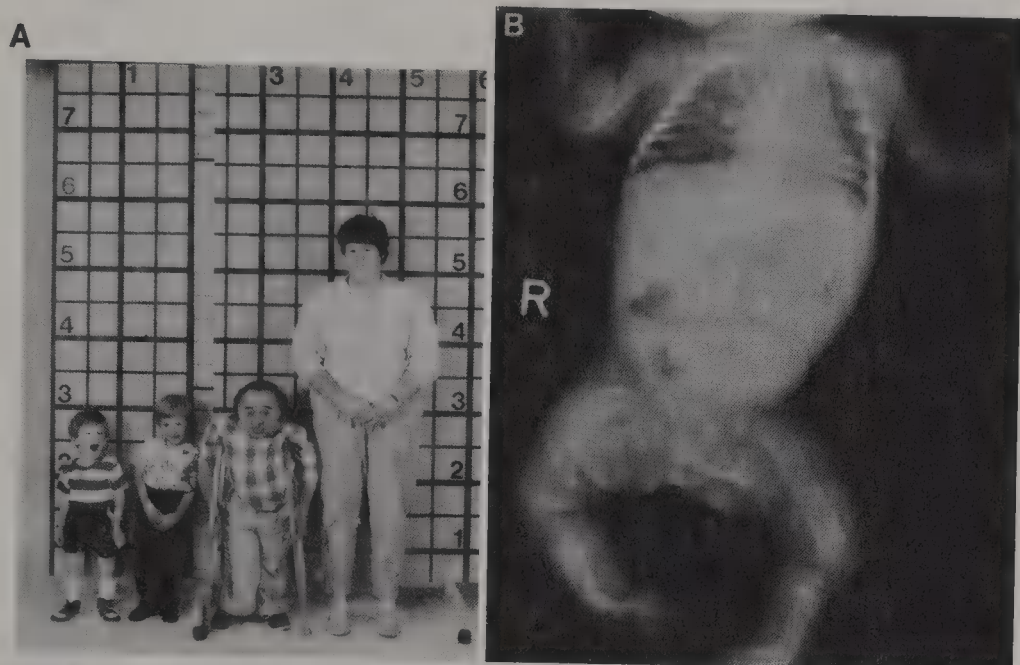


FIG 8. Cases of OI with unexpected inheritance. **A**, man with progressive deforming OI and his normal spouse have two sons, ages 4 and 7 years old, with type IV-like OI. **B**, radiograph of 5-month-old child with severe OI. Her father is affected with progressive deforming OI and had a sibling who died from OI in infancy.

represent genetic compounds or the extreme range of that family's variability. Furthermore, an infant with perinatal lethal OI has been reported in a mild type I family.⁶⁴ For dominant pedigrees with several generations of affected members, a study of collagen RFLPs may be useful in determining which clinically ambiguous family members are in fact carriers. For sporadic type IV cases in which the proband has overmodified collagen and the parents do not, the empirical recurrence risk is generally low (3% to 5%). In summary, when counselling the parents of a child with clinical type I or IV disease, one needs to carefully examine the pedigree, the clinical status of the parents, and in some cases the molecular biology of the nuclear family.

Prenatal Diagnosis

Early prenatal diagnosis of OI is useful because it permits couples to make a choice concerning the continuation or termination of the pregnancy. If the parents choose to continue an affected pregnancy, plans can then be made for delivery by elective cesarean section to optimize the infant's chances of survival. The risks of normal vaginal delivery include evulsion of fragile body parts, intracranial hemorrhage, and fractures of thoracic cage and long bones.

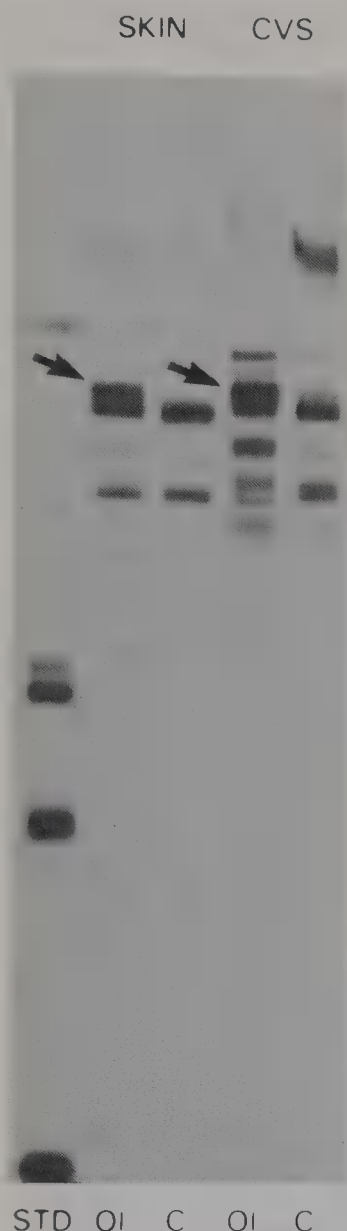
Prenatal diagnosis of type II can be achieved by ultrasound detection of deformed long bones with poor echogenic properties, as well as skull and thorax abnormalities. Numerous cases have been detected at 17 to 20 weeks' gestation.⁶⁵⁻⁶⁹ When a random screening ultrasound detects a fetus compatible with the diagnosis of severe OI, the differential diagnosis includes hypophosphatasia as well as other forms of dwarfism. Ultrasound detections of progressive deforming OI have been reported at 19 to 20 weeks' gestation.^{70, 71} Occasionally an infant from a milder dominant pedigree may be detected by demonstrating an in utero fracture.

For dominant pedigrees that are informative for one of the type I collagen RFLPs, there is the possibility of prenatal diagnosis by Southern blot analysis of DNA extracted from chorionic villi.⁷² For the test to be useful, the DNA extracted from the white blood cells of both unaffected and affected individuals in several generations must be tested in advance. The statistical accuracy of the prediction varies from family to family, depending on the tightness of the linkage between the RFLP and the disease phenotype. Sporadic cases and noninformative pedigrees cannot be offered this testing.

More recently, several groups (Marini, Byers, and Cole, unpublished data) have been exploring the feasibility of prenatal diagnosis using cultured cells from chorionic villus sampling (CVS). Collagen studies using cultured cells from CVS show the same defect as do the cultured fibroblasts from an affected fetus (Fig 9). While this is still a research test, defects involving overmodification seem to be detectable. The CVS would be done at 8 to 10 weeks' gestation. Adding the time required for culturing cells and the actual collagen studies, the results could be available at about 16 weeks' gestation. Follow-up ultrasound should be performed on any pregnancy tested for OI by CVS. For a couple concerned about recurrence of type II, CVS offers an earlier diagnosis than ultrasound with a small risk of fetal loss. Testing via CVS probably has its greatest utility for moderately severe or mild cases where late detection by ultrasound might preclude a therapeutic abortion for parents desiring this option. In the future, CVS will probably be used to provide tissue for probes that detect the mutation directly at the DNA or RNA level.

Clinical Management

There is at present no treatment or cure for OI. Orthopedic treatment of fractures and skeletal deformity remains essential to clinical management. However, many severely affected OI children can achieve greater motor function with a program that includes aggressive physical medicine than with surgical procedures alone. Because of the multisystem involvement associated with OI, a multidisciplinary team is essential for complete clinical management. Even children with mild disease deserve periodic evaluation at a center offering multisystem evaluation.

**FIG 9.**

SDS gel demonstrating detection of type I collagen abnormality in cultured chorionic villi from case shown in Fig 1. The skin and CVS of the infant are each compared with control cells (C) from the same tissues. Arrows show the detection of the overmodified chain in skin and CVS.

Review of Pharmacologic Therapies

The two drugs whose effect on OI have been studied most thoroughly are calcitonin and fluoride. The logic behind the attempts at calcitonin therapy, pioneered by Castells et al.,^{73, 74} was that its direct inhibitory effect on osteoclastic resorption of both the mineral and the matrix phases of bone could be exploited to increase bone density. Treatment of two children with type I with porcine calcitonin was reported to be associated with changes in calcium, phosphorus, and hydroxyproline balance. Forty-eight

children were treated with salmon calcitonin for 1 to 2 years; a decreased fracture rate and increase in hand bone density were reported. However, (1) calcitonin has significant side effects on electrolyte balance; (2) rib biopsy specimens before and after 1 year of therapy fail to show any histologic changes⁷⁵; and (3) data on normal bone suggest that bone chronically exposed to calcitonin adjusts to its inhibitory effect.

Sodium fluoride is a component of the armamentarium used to treat postmenopausal osteoporosis, where its greatest effect has been demonstrated to be on the trabecular bone of vertebral bodies and hips.⁷⁶ In the bone, fluoride substitutes itself isomorphically for hydroxyl ions and the resulting matrix composition is fluorohydroxyapatite. The therapeutic window of fluoride is relatively narrow; high doses cause significant bone sclerosis. Fluoride's positive effect on trabecular bone has not been demonstrated for the cortical bone of the appendicular skeleton.⁷⁶ Furthermore, the inhibitory effect of fluoride on osteoclasts might interfere with bone remodelling. There have been anecdotal reports of fluoride therapy in OI⁷⁷ but no carefully controlled trials. Fluoride may have its best use in preventing the vertebral compression fractures common in adults with OI.

Agents that stimulate procollagen synthesis by osteoblasts, such as ascorbate or vitamin D, are logical candidates for clinical trials in those OI patients who secrete reduced amounts of apparently normal collagen. The flavinoid Categeren has been shown to affect collagen structure by its effect on lysyl hydroxylase activity. In one report,⁷⁸ the diameter of collagen fibrils in bone from an affected adult was significantly improved by 6 months of therapy. However, the long bones of his affected sons showed less response; neither the adult nor his children showed any change in vertebral bone density.

Rehabilitation Medicine

The goal of rehabilitation medicine in OI is the maximization of the age-appropriate activities and skills of the child; this goal includes standing or short-distance ambulation, even in most moderately severe cases. The goals and methods summarized here represent the philosophy of an ongoing collaborative study between the National Institute of Child Health and Human Development, the NIH Clinical Center Department of Rehabilitation Medicine, and Children's Hospital National Medical Center (CHNMC), Washington, DC. Results of such an aggressive rehabilitation approach⁷⁹ to children with moderately severe and progressively deforming OI are encouraging, and a controlled study is planned. Its long-term effects on ambulation, osteoporosis, and disease morbidity are unknown. Many clinics treating severe OI patients place less emphasis on ambulation as a goal.

Many clinics also place little emphasis on physical therapy. However, a physical therapist trained in handling these children can be extremely help-

ful to child and parents, beginning in the neonatal period. Even severely involved infants should be positioned properly to prevent head and spinal deformities, improve their breathing, and minimize fractures. Swimming is the safest exercise for all ages (Fig 10,A) and can begin in the first few weeks of life in the bath tub. The buoyancy of the water permits active movement of the extremities and aids in the development of head and trunk control, as well as strengthening the muscles of the extremities. Infants who are not expected to walk should be positioned in a custom-molded seat to align the head, spine, and pelvis optimally. Those who have potential for standing and walking should receive vigorous physical

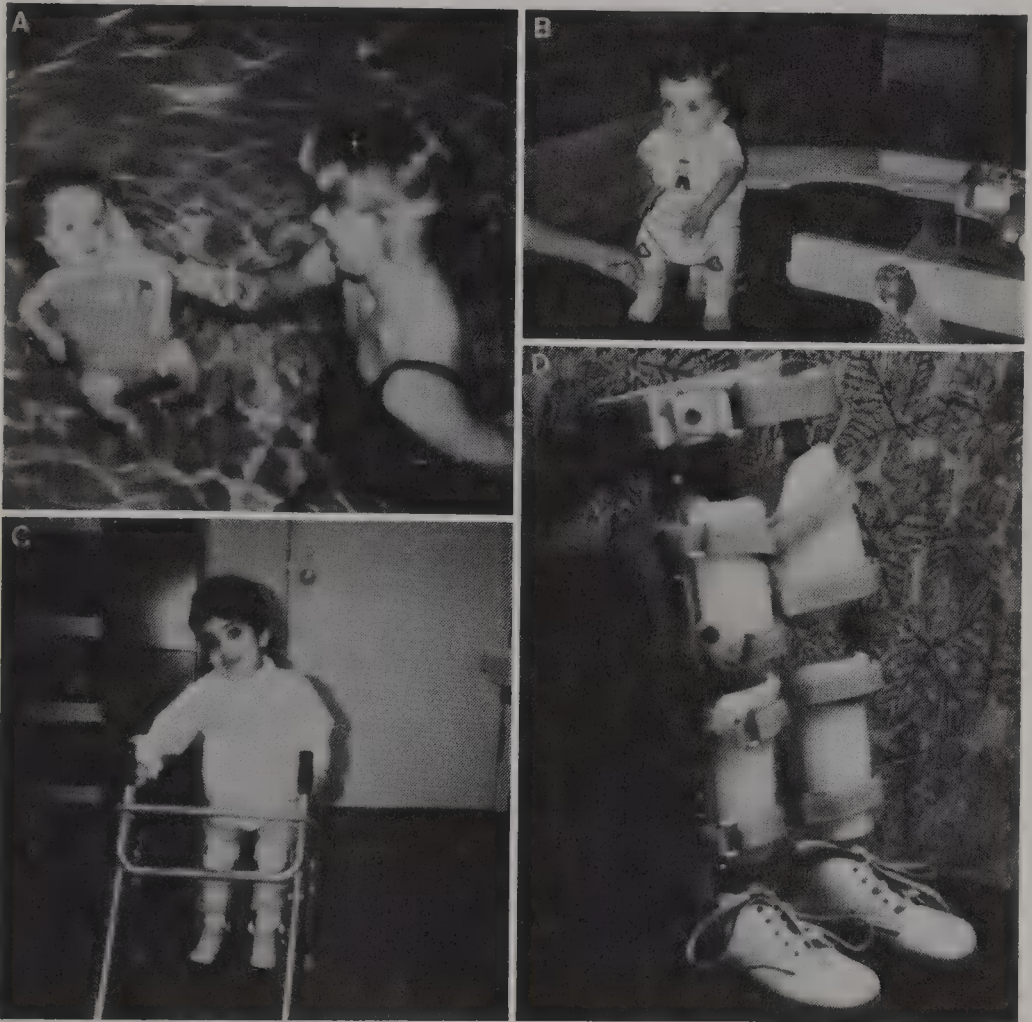


FIG 10.

Rehabilitation of children with OI. **A**, 1-year-old infant with severe OI swimming with mother. **B**, 3-year-old child in standing frame. Note play table made by patient's family. **C**, 5-year-old boy walking with braces and walker. **D**, example of braces used for ambulation.

therapy to prevent hip flexion and abduction contractures and to strengthen their abdominal, pelvic girdle, and lower extremity muscles.

Later, usually in the second or third year of life, these children are fitted for braces and unsupported walking is discouraged. Some children require surgery to decrease lower long bone deformity before standing is attempted. In the United States, Bleck⁸⁰ pioneered the use of the containment-type lightweight braces. The NIH-CHNMC program uses ultra-lightweight plastic clam-shell braces for ischial weight-bearing and, when appropriate, hinged joints (see Fig 10,D). A consistent home program of swimming and physical therapy is an essential component of the program. Approximately a dozen children who would otherwise have a poor prognosis for ambulation are active in these braces (see Fig 10,B and C) and interact more normally with their peers. Fractures do occur in the braces, but are often reduced in frequency from the child's pretreatment fracture rate. Because of their short stature, many of these children would be household ambulators and use wheelchairs for distance mobility. Nonetheless, having some degree of ambulation ability will make these individuals less handicapped and more independent adults.

Orthopedic Management

Orthopedic care by a surgeon with experience in the treatment of OI is a key component of management. If fractures are allowed to heal without any attempt at alignment or reduction, the child will lose considerable function. In general, a fracture should be managed with traction or casts as would a similar fracture in a normal child, except that the period of immobilization should be kept as short as possible to avoid worsening the osteoporosis.

If fractures are especially frequent, a vicious cycle of fracturing, deformity, osteoporosis, and re-fracturing may occur. Even without fractures, the pull of strong lower extremity muscles on bones and the weight of the the trunk can cause considerable anterior bowing deformity. It is the purpose of various osteotomy procedures to correct bowing deformities and prevent bone fractures. The classic Sofield and Millar procedure of multiple osteotomies, realignment, and intramedullary rod fixation has been used in multiple patients,⁸¹ usually for lower extremities. Since its introduction in 1963, this procedure has often been done with the Bailey-Dubow extensible rod,⁸² which traverses the bone from end to end (Fig 11,B). Since it extends with bone growth, it avoids the problem of the bone overgrowing the rod. The time between rod changes (4.0 versus 2.5 years) is increased by use of these rods.⁸³ However, it has no advantage in the prevention of fractures or the recovery of walking. Longitudinal migration of the ends of the rod is a not infrequent complication of extensible rods. Migration into a joint space necessitates rod removal. Unfortunately, there is a price to be paid for the stabilization provided by intramedullary rods.

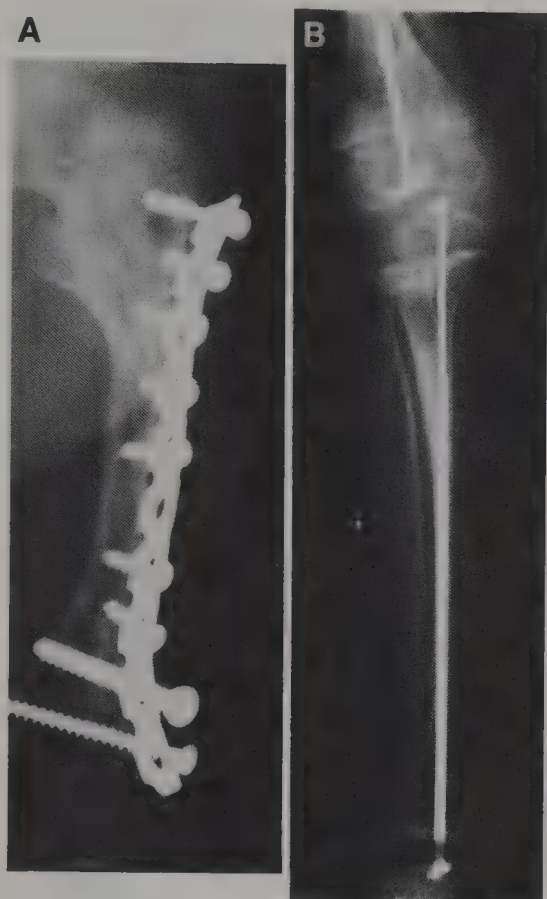


FIG 11.

Orthopedic appliances used in OI. **A**, plate and screws used to stabilize osteoporotic femur. **B**, Bailey-Dubow rods in lower long bones of boy shown in Figure 2,A. Note the shrinkage of the tibial shaft around the rod and the very thin cortex.

Significant cortical atrophy, particularly in the diaphysis, occurs (see Fig 11,B).⁸⁴ An alternative procedure for the correction of deformity and the prevention of fractures is the use of a plate and screws (see Fig 11,A). Anecdotal experience reports fewer fractures postsurgically if a long plate is used to cover the entire bone. It is hoped that the amount of stress unloading and cortex shrinkage may be less than with intramedullary rods.

For severely affected infants, Middleton^{85, 86} pioneered the use of manual osteoclasis and fixation by percutaneous intramedullary rods. Solid So-field rods are inserted under x-ray image intensification. In fragile, young infants, this procedure requires a relatively short operative time and avoids bone exposure. In the NIH-CHNMC collaborative effort, temporary percutaneous rods have been used to straighten lower extremities prior to bracing.

The progressive scoliosis of severe OI does not respond to conservative management with Milwaukee bracing.^{87, 88} Resultant deformities of the thorax adversely affect pulmonary function. In several series of patients treated by spinal fusion with Harrington instrumentation, the rate of complications is high^{88, 89} but stabilization of the fused spine is generally good.

Neurologic Evaluation

In cases of severe OI, the neurologic examination may be abnormal due to bony compression of the cervical spinal cord. A constricted V-shaped foramen magnum causing upper cervical cord compression has been reported.⁹⁰ Pozo and colleagues⁹¹ reviewed reported cases of basilar impression in mild OI.

Tsipouras et al.⁹² reported the computed tomography (CT) scans of ten OI patients. Four type I patients, three with macrocephaly, had normal CT scans. The CT scans of six patients with type III were abnormal. Ventriculomegaly and sulcal prominence, interpreted as cortical atrophy, were found. We evaluated the neurologic examination and selected CT scans (Charnas, Marini, Hurko; manuscript in preparation) of 20 children with OI in the NIH bracing protocol. Ventriculomegal and sulcal prominence were common findings, even in children without absolute macrocephaly (Fig 12). Like Tsipouras et al., we feel that this is a characteristic OI finding and does not indicate active hydrocephalus. However, we caution the interpretation of these CT scans as "atrophy."

Additionally, in severe OI cases we have seen odontoid hypoplasia and odontoid cervicomedullary compression in patients evaluated because of long tract signs. All our cases appeared to be stable for routine daily activities. Precautions should be taken so that such children do not have neck hyperextension during intubation for anesthesia.

Ocular Findings

The most common ocular finding in OI is blue sclerae. The blueness, which is often blue-grey in hue, is often less intense near the limbus. There are numerous anecdotal reports of scleral hue deepening around the time of a fracture.²⁰ Blue sclerae are not limited to OI patients; they are seen in other disorders with primary or secondary connective tissue manifestations. Occasionally, persons without connective tissue symptoms present with markedly blue sclerae.

It had been thought that the blue color resulted from transmission of the color of the choroid through thin sclerae.²⁰ Lanting and co-workers⁹³ presented evidence that the blueness may result from a decreased scattering coefficient rather than from thinness per se. Such intrinsic factors as water content, mucopolysaccharide content, or collagen fiber diameter and packing could affect the scattering coefficient. The Chan et al. report⁹⁴ on the histopathology of blue sclerae at autopsy notes marked reduction in collagen fiber diameter and packing in OI sclerae as well as relative thinness of the sclerae.

Kaiser and co-workers^{95, 96} showed that ocular rigidity, measured as distensibility of the coats of the eye when the cornea is indented with a Schiotz tonometer, is significantly lower in OI patients than in normal con-



FIG 12.

CT scans of OI children. **A** and **D** show the CT scans of the 10-month-old twins with severe OI shown in **B**. Note ventriculomegaly and sulcal prominence. **C** is a CT scan of the boy shown in Figure 3, **B**. Note prominent ventricular size.

trols. There was a statistically significant correlation between decreasing ocular rigidity and increasing blueness of sclerae; low ocular rigidity was not related to a myopic refractive error. Furthermore, these data do not imply that blue sclerae have a decreased tensile strength or are more easily ruptured.

Other ocular findings reported in OI are decreased central corneal thickness,²⁰ keratoconus, and premature arcus senilis.

Hearing Impairment

Hearing loss is present in a significant proportion of patients with type I (variously reported as 25% to 60%) and often represents their most signif-

icant handicap. Clinically significant hearing loss is usually not noted before the patient is in his or her 20s, although audiologic abnormalities are often present earlier. The proportion of affected individuals rises steadily into middle age, despite generally stable skeletal disease in these individuals. Pedersen⁹⁷ surveyed 201 Danish OI patients and found 39% with a conductive or mixed hearing loss and 11% with a sensorineural loss. Tympanometry and acoustic reflexes were suggestive of stapedial fixation. Riedner et al.⁹⁸ studied 13 families with dominant OI using impedance audiometry and found an increasing sensorineural component of the hearing loss with increasing age. The survey of Shapiro et al.,⁹⁹ using otoadmittance, found a high proportion of sensorineural loss in a younger age group and considered a mild high frequency loss to be characteristic of OI. The audiologic examination of unaffected relatives of patients in this survey needs to be reappraised with genetic and biochemical correlates.

Although the clinical otologic pictures of otosclerosis and OI are very similar, hearing loss in OI occurs earlier and has more severe middle ear involvement. Furthermore, the histologic pattern is distinct¹⁰⁰⁻¹⁰² with the OI stapes footplate displaying immature woven bone, a much greater area of resorptive surface, and fewer capillaries than with otosclerosis.

Several surgical series^{100, 103-105} suggest that stapedectomy can give satisfactory long-term results for OI patients with severe hearing loss. Intraoperatively, care must be taken to avoid fracturing the fragile stapes or creating a "floating footplate." This surgery should not be considered routine in OI patients. When a hearing aid does not provide adequate amplification, referral to a center with OI experience is recommended.

Dentinogenesis Imperfecta

Dental abnormalities have long been recognized as part of OI. In DI (see Fig 4), the teeth may be greyish, bluish, or brown and opalescent. Although the teeth have normal enamel, they may also crumble and fracture. The primary dentition is generally more severely abnormal than the permanent dentition. The variability of dental findings in OI has been reviewed.¹⁰⁶⁻¹⁰⁸ DI also occurs separately from OI.

On scanning electron microscopy, there are fewer dentin tubules in teeth with DI than controls and the calcification fronts are composed of irregular, small nodules.¹⁰⁹ In normal dentin, immunohistochemical stains have shown virtual absence of type III collagen from intertubular dentin.¹¹⁰ The role of altered type I/type III ratios in the pathogenesis of DI is unknown.

Levin et al.¹¹ reported the consistency of DI in pedigrees with dominant OI. Within a given family, DI is present in all or none of the persons affected with OI. In families with OI and DI, DI may be the only symptom of an individual carrying the OI gene. On this basis, the dominant forms of OI have been subdivided into types with and without DI. This division represents a presumptive molecular grouping of the gene defects in OI.

Other oral abnormalities reported in OI include pulp chamber occlusion and malocclusion.^{108, 111} Three families with dominant OI without DI had multilocular radiolucent or radiopaque lesions of the maxilla and mandible, usually involving tooth-bearing areas.¹¹² The youngest such case was noted at 8 years of age.

Children with DI should be seen by their dentist at least twice yearly. Artificial crowns may be necessary to preserve tooth structure.¹⁰⁶ With the advent of new dental restorative materials, coating of the teeth may be adequate to preserve tooth structure in milder cases.

Cardiovascular Manifestations

Although mitral valve prolapse does not appear to be more prevalent among all individuals with OI than in the general population (5% to 7%), it does tend to be part of the syndrome in dominant pedigrees. Affected relatives of an affected individual with mitral valve prolapse should be examined and prophylaxed appropriately. Aortic elastin was reported to be abnormal in a case of perinatal lethal OI.¹¹³ Elastin fibers in the aorta were thicker and less homogeneous than controls, although skin elastin appeared normal. In a series¹¹⁴ of adults with mild, dominant OI, one third had increased aortic wall stiffness (in contrast to the Marfan Syndrome where the aorta has decreased stiffness). Dilatation of the aortic ring and aortic regurgitation are also reported.²⁰

Many OI patients exhibit easy bruising. Hemostasis was studied in 58 patients with mild, dominant OI.¹¹⁵ One third of patients exhibited capillary fragility in the tourniquet test, while the bleeding time was normal in 90%.

Growth Deficiency

Growth impairment is one of the more dramatic secondary effects of OI, affecting virtually 100% of type III patients and 25% to 50% of types I and IV. No simple correlation exists between fracture frequency or location and growth failure. In an NICHD protocol (Marini et al., manuscript in preparation), 20 children underwent extensive testing of the growth hormone–somatomedin axis to determine whether hormonal deficits are associated with growth failure in OI. Evaluation included growth hormone provocative tests, serial sampling of growth hormone over a 24-hour period to determine the unstimulated human growth hormone secretion, and somatomedin generation in response to growth hormone. Two patterns of hormone abnormality were found: (1) neurosecretory deficiency of growth hormone with normal provocative tests and somatomedin generation and (2) normal growth hormone secretion but low baseline somatomedin levels that fail to respond in a somatomedin generation test. The cause of this secondary feature of OI is not clear. However, preliminary results of a pilot

study in which OI children with neurosecretory deficiency of growth hormone were treated with clonidine or protropin were encouraging.

Summary and Future Prospects

Defects in either of the type I collagen proteins have been demonstrated in some cases of OI. Using the Sillence classification and the results of molecular studies, genetic counselling is more accurate and often more optimistic about recurrence risks. Prenatal diagnosis, through ultrasound or CVS, is available for many cases. More aggressive management of severe OI cases, using a multidisciplinary approach, holds the prospect of greater general health and skeletal function.

However, many aspects of OI await clarification. The most intriguing question is the relationship of specific mutations to the pathogenesis of the disease. Other areas of future molecular and clinical research include (1) whether collagen gene defects are present in all cases of OI, (2) the basis for the dramatic variability in OI phenotype in some families and its relationship to type I collagen and other bone matrix proteins, (3) the role of gonadal mosaicism and compound heterozygotes in connective tissue disease, (4) improved prenatal diagnosis, and (5) the feasibility of modifying clinical features of OI such as osteoporosis and growth deficiency. The answers to these questions will not only enable us to assist patients with connective tissue disorders but will also enhance our understanding of the structural/functional biology of bone matrix.

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References

1. Gray PHK: A case of osteogenesis imperfecta, associated with dentinogenesis imperfecta, dating from antiquity. *Clin Radiol* 1970; 21:106.
2. Ekman O: Dissertatio medica descriptionem et casus aliquot osteomalaciae sistens. *Upsala*, 1788.
3. Vrolik W: Tabulae ad illustrandam embryogenesis hominis et mammalium, tam naturalem quam abnormen. *Amstelodami*, 1849.

4. Looser E: Zur kenntnis der osteogenesis imperfecta congenita et tarda. *Mitt Girenzgeb Med Chir* 1906; 15:161.
5. Van der Hoeve J, de Kleyn A: Blaue sclerae, knochenbruchigkeit und schwerhörigkeit. *Arch Ophthal* 1918; 95:81.
6. Sillence DO, Senn A, Danks DM: Genetic heterogeneity in osteogenesis imperfecta. *J Med Genet* 1979; 16:101.
7. Sillence DO: Osteogenesis imperfecta: An expanding panorama of variants. *Clin Ortho Rel Res* 1981; 159:11.
8. Sillence DO, Barlow KK, Garber AP, et al: Osteogenesis imperfecta type II: Delineation of the phenotype with reference to genetic heterogeneity. *Am J Med Genet* 1984; 17:407.
9. Sillence DO, Barlow KK, Cole WG, et al: Osteogenesis imperfecta type III: Delineation of the phenotype with reference to genetic heterogeneity. *Am J Med Genet* 1986; 23:821.
10. Spranger J: Osteogenesis imperfecta: A pasture for splitters and lumpers. *Am J Med Genet* 1984; 17:425.
11. Levin LS, Salinas CF, Jorenson RJ: Classification of osteogenesis imperfecta by dental characteristics. *Lancet* 1978; 1:332.
12. Horton WA, Dockery H, Sillence DO, et al: An embedding method for histochemical studies of undecalcified skeletal growth plate. *Stain Technol* 1980; 55:19.
13. Ornoy A, Kim OJ: Scanning electron microscopy findings in osteogenesis imperfecta fetalis. *Israel J Med Sci* 1977; 13:26.
14. Paterson CR, McAllion S, Miller R: Heterogeneity of osteogenesis imperfecta type I. *J Med Genet* 1983; 20:203.
15. Bergstrom L: Osteogenesis imperfecta: otologic and maxillo-facial aspects. *Laryngoscope* 1977; 87(suppl 6):1.
16. de Vries WN, de Wet WJ: The molecular defect in an autosomal dominant form of osteogenesis imperfecta. *J Biol Chem* 1986; 261:9056.
17. Paterson CR, McAllian S, Miller R: Osteogenesis imperfecta with dominant inheritance and normal sclerae. *J Bone Joint Surg (Am)* 1982; 6:35.
18. Wenstrup RJ, Tspouras P, Byers PH: Osteogenesis imperfecta type IV. *J Clin Invest* 1986; 78:1449.
19. Wynne-Davies R, Gormley J: Clinical and genetic patterns in osteogenesis imperfecta. *Clin Orthop Rel Res* 1981; 159:26.
20. McKusick VA: Osteogenesis imperfecta, in *Heritable Disorders of Connective Tissue*, ed 4. St Louis, CV Mosby Co, 1972.
21. Prockop DJ, Kivirikko KI, Tuderman L, et al: The biosynthesis of collagen and its disorders. *N Engl J Med* 1979; 301:13(part I), 77 (part II).
22. Prockop DJ, Kivirikko KI: Heritable diseases of collagen. *N Engl J Med* 1984; 311:376.
23. Pope FM, Nicholls AC, McPheat J, et al: Collagen genes and proteins in osteogenesis imperfecta. *J Med Genet* 1985; 22:466.
24. Hollister DW: Molecular basis of osteogenesis imperfecta. *Curr Probl Derm* 1987; 17:76.
25. Huerre C, Junien C, Weil D, et al: Human type I procollagen genes are located on different chromosomes. *Proc Natl Acad Sci USA* 1982; 79:6627.
26. Junien C, Weil D, Myers JC: Assignment of the human pro $\alpha 2(I)$ collagen structural gene (COL1A2) to chromosome 7 by molecular hybridization. *Am J Hum Genet* 1982; 34:381.

27. Yamada Y, Avvedimento EV, Mudrji M, et al: The collagen gene: Evidence for its evolutionary assembly by amplification of a DNA segment containing an exon of 54 bp. *Cell* 1980; 22:887.
28. Wozney J, Hanahan D, Tate V, et al: Structure of the pro $\alpha 2(I)$ collagen gene. *Nature* 1981; 294:129.
29. Chu M-L, Myers JC, Bernard MP, et al: Cloning and characterization of five overlapping cDNAs specific for the human pro $\alpha 1(I)$ collagen chain. *Nucl Acids Res* 1982; 10:5925.
30. Barsh GS, Roush CL, Gelinas RE: DNA and chromatin structure of the human $\alpha 1(I)$ collagen gene. *J Biol Chem* 1984; 259:14906.
31. Myers JC, Dickson LA, de Wet WJ, et al: Analysis of the 3' end of the human pro $\alpha 2(I)$ collagen gene: Utilization of multiple polyadenylation sites in cultured fibroblasts. *J Biol Chem* 1983; 258:10128.
32. Kivirikko KI, Myllyea R: Hydroxylation of prolyl and lysyl residues, in Freedman RB, Hawkins HC (eds): *The Enzymology of Post-translational Modification of Proteins*. London, London Academic Press, 1980, p 53.
33. Kivirikko KI, Myllyea R: Collagen glycosyltransferases. *Int Rev Connect Tiss* 1979; 18:23.
34. Tuderman L, Prockop DJ: Procollagen N-proteinase: Properties of the enzyme purified from chick embryo tendons. *Eur J Biochem* 1982; 125:545.
35. Williams CJ, Prockop DJ: Synthesis and processing of a type I procollagen containing shortened pro $\alpha 1(I)$ chains by fibroblasts from a patient with osteogenesis imperfecta. *J Biol Chem* 1983; 258:5915.
36. Chu M-L, Williams CJ, Pepe G, et al: Internal deletion in a collagen gene in a perinatal lethal form of osteogenesis imperfecta. *Nature* 1983; 304:78.
37. Barsh GS, Rouse CL, Bonadio J, et al: Intron-mediated recombination may cause a deletion in an $\alpha 1$ type I collagen chain in a lethal form of osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1985; 82:2870.
38. de Wet WJ, Pihlajaniemi T, Myers J, et al: Synthesis of a shortened pro $\alpha 2(I)$ chain and decreased synthesis of pro $\alpha 2(I)$ chains in a proband with osteogenesis imperfecta. *J Biol Chem* 1983; 258:7721.
39. Steinman B, Rao VH, Vogel A, et al: Cysteine in the triple-helical domain of one allelic product of the $\alpha 1(I)$ gene of type I collagen produces a lethal form of osteogenesis imperfecta. *J Biol Chem* 1984; 259:11129.
40. Bateman JF, Chan D, Walker ID, et al: Lethal perinatal osteogenesis imperfecta due to the substitution of arginine for glycine at residue 391 of the $\alpha 1(I)$ chain of type I collagen. *J Biol Chem* 1987; 262:7021.
41. Bateman JF, Chan D, Mascara T, et al: Collagen defects in lethal perinatal osteogenesis imperfecta. *Biochem J* 1986; 240:699.
42. Bonadio J, Byers PH: Subtle structural alterations in the chains of type I procollagen produce osteogenesis imperfecta Type II. *Nature* 1985; 316:363.
43. Bateman JF, Mascara T, Chan D, et al: Abnormal type I collagen metabolism by cultured fibroblasts in lethal perinatal osteogenesis imperfecta. *Biochem J* 1984; 217:103.
44. Prockop DJ: Osteogenesis imperfecta: Phenotypic heterogeneity, protein suicide, short and long collagen. *Am J Hum Genet* 1984; 36:499.
45. Dickson LA, Pihlajaniemi T, Deak S, et al: Nuclease S1 mapping of a homozygous mutation in the carboxyl-propeptide-coding region of the pro $\alpha 2(I)$ collagen gene in a patient with osteogenesis imperfecta. *Proc Natl Acad Sci USA* 1984; 81:4524.

46. Pihlajaniemi T, Dickson LA, Pope FM, et al: Osteogenesis imperfecta: Cloning of a pro $\alpha 2(I)$ collagen gene with a frame shift mutation. *J Biol Chem* 1984; 259:12941.
47. Deak SB, Nicholls A, Pope FM, et al: The molecular defect in a non lethal variant of osteogenesis imperfecta. *J Biol Chem* 1983; 258:15192.
48. Sippola M, Kaffe S, Prockop DJ: A heterozygous defect for structurally altered pro $\alpha 2$ chain of type I procollagen in a mild variant of osteogenesis imperfecta. *J Biol Chem* 1984; 259:14094.
49. Rowe DW, Shapiro JR, Schlesinger S: Diminished type I collagen synthesis and reduced $\alpha 1(I)$ collagen messenger RNA in cultured fibroblasts from patients with dominantly inherited (type I) osteogenesis imperfecta. *J Clin Invest* 1985; 76:604.
50. Barsh GS, David KE, Byers PH: Type I osteogenesis imperfecta: A nonfunctional allele for pro $\alpha 1(I)$ chains of type I procollagen. *Proc Natl Acad Sci USA* 1982; 79:3838.
51. Nicholls AC, Pope FM, Craig D: An abnormal collagen α chain containing cysteine in autosomal dominant osteogenesis imperfecta. *Br Med J* 1984; 288:112.
52. Tsipouras P, Borresen A-L, Dickson LA, et al: Molecular heterogeneity in the mild autosomal dominant forms of osteogenesis imperfecta. *Am J Hum Genet* 1984; 36:1172.
53. Sykes B, Ogilvie D, Wordsworth P, et al: Osteogenesis imperfecta is linked to both type I collagen structural genes. *Lancet* 1986; 12:69.
54. Harbers K, Kuehn M, Delius H, et al: Insertion of retrovirus into the first intron of $\alpha 1(I)$ collagen gene leads to embryonic lethal mutation in mice. *Proc Natl Acad Sci USA* 1984; 81:1504.
55. Lohler J, Timpl R, Jaenisch R: Embryonic lethal mutation in mouse collagen $\alpha 1$ gene causes rupture of blood vessels and is associated with erythropoietic and mesenchymal cell death. *Cell* 1984; 38:597.
56. Stacey A, Mulligan R, Jaenisch R: Rescue of type 1 collagen deficient phenotype by retroviral-vector-mediated transfer of human pro $\alpha 1(I)$ collagen gene into Mov-13 cells. *J Virol* 1987; 61:2549.
57. Schnieke A, Dziadek M, Bateman J, et al: Introduction of the human pro $\alpha 1(I)$ collagen gene into pro $\alpha 1(I)$ deficient Mov-13 mouse cells leads to formation of functional mouse-human hybrid type I collagen. *Proc Natl Acad Sci USA* 1987; 84:764.
58. Steinman B, Nicholls A, Pope FM: Clinical variability of osteogenesis imperfecta reflecting molecular heterogeneity: Cysteine substitutions in the $\alpha 1(I)$ collagen chain producing lethal and mild forms. *J Biol Chem* 1986; 261:8958.
59. Muller PK, Raisch K, Matzen K, et al: Presence of type III collagen in bone from a patient with osteogenesis imperfecta. *Eur J Pediatr* 1977; 125:29.
60. Pope FM, Nicholls AC, Eggleton C, et al: Osteogenesis imperfecta (lethal) bones contain types III and V collagens. *J Clin Pathol* 1980; 33:534.
61. Minor RR, Wootton JAM, Prockop DJ, et al: Genetic diseases of connective tissue in animals. *Curr Probl Derm* 1987; 17:199.
62. Pope FM, Nicholls AC, Paterson CR: Heterogeneity of osteogenesis imperfecta congenita. *Lancet* 1980; 12:820.
63. Carothers AD, McAllion SJ, Paterson CR: Risk of dominant mutation in older fathers: Evidence from osteogenesis imperfecta. *J Med Genet* 1986; 23:227.

64. Gillerot Y, Druart JM, Koulischer L: Lethal perinatal type II osteogenesis imperfecta in a family with a dominantly inherited type I. *Eur J Pediatr* 1983; 141:119.
65. Shapiro JE, Phillips JA, Byers PH, et al: Prenatal diagnosis of lethal perinatal osteogenesis imperfecta (OI type II). *J Peds* 1982; 100:127.
66. Chervenak FA, Romero R, Berkowitz RL, et al: Antenatal sonographic findings of osteogenesis imperfecta. *Am J Obstet Gynecol* 1982; 143:228.
67. Ghosh A, Woo JSK, Wan CW, et al: Simple ultrasonic diagnosis of osteogenesis imperfecta type II in early second trimester. *Prenat Diag* 1984; 4:235.
68. Stephens JD, Filly RA, Callen PW, et al: Prenatal diagnosis of osteogenesis imperfecta type II by real-time ultrasound. *Hum Genet* 1983; 64:191.
69. Elejalde BR, Elejalde MM: Prenatal diagnosis of pernatally lethal osteogenesis imperfecta. *Am J Med Genet* 1983; 14:353.
70. Ayesworth AS, Seeds JW, Guilford WB, et al: Prenatal diagnosis of a severe deforming type of osteogenesis imperfecta. *Am J Med Genet* 1984; 19:707.
71. Robinson LP, Worthen NJ, Lachman RS, et al: Prenatal diagnosis of osteogenesis imperfecta type III. *Prenat Diag* 1987; 7:7.
72. Tsipouras P, Schwartz RC, Goldberg JD, et al: Prenatal prediction of osteogenesis imperfecta (OI type IV): Exclusion of inheritance using a collagen gene probe. *J Med Genet* 1987; 24:406.
73. Castells S, Inamdar S, Baker RK, et al: Effects of porcine calcitonin in osteogenesis imperfecta tarda. *J Peds* 1972; 80:757.
74. Castells S, Colbert C, Chakrabart C, et al: Therapy of osteogenesis imperfecta with synthetic salmon calcitonin. *J Peds* 1979; 95:807.
75. August GP, Shapiro J, Hung W: Calcitonin therapy of children with osteogenesis imperfecta. *J Peds* 1980; 91:1001.
76. Riggs BL: Treatment of osteoporosis with sodium fluoride: An appraisal. *Bone Mineral Res* 1983; 2:366.
77. Aeschlimann MI, Grunt JA, Crigler JF: Effects of sodium fluoride on the clinical course and metabolic balance of an infant with osteogenesis imperfecta congenita. *Metabolism* 1966; 15:905.
78. Jones CJ, Cummings C, Ball J, et al: A clinical and ultrastructural study of osteogenesis imperfecta after flavonoid (catergen) therapy. *SA Med J* 1984; 66:907.
79. Binder H, Hawks L, Graybill G, et al: Osteogenesis imperfecta: Rehabilitation approach with infants and young children. *Arch Phys Med Rehab* 1984; 65:537.
80. Bleck E: Nonoperative treatment of osteogenesis imperfecta: Orthotic and mobility management. *Clin Orthop Rel Res* 1981; 159:111.
81. Sofield HA, Millar EA: Fragmentation, realignment and intramedullary rod fixation of deformities of the long bones in children: A ten-year appraisal. *J Bone Joint Surg (Am)* 1959; 41:1371.
82. Bailey RW, Dubow HI: Studies of longitudinal bone growth resulting in an extensible nail, in *Surgical Forum*. Chicago, American College of Surgeons, vol XIV, 1963.
83. Marafioti RL, Westin GW: Elongating intramedullary rods in the treatment of osteogenesis imperfecta. *J Bone Joint Surg* 1977; 59:467.
84. Millar EA: Observations on the surgical management of osteogenesis imperfecta. *Clin Orthop Rel Res* 1980; 159:154.
85. Middleton RWD: Closed intramedullary rodding for osteogenesis imperfecta. *J Bone Joint Surg (Br)* 1984; 66:652.

86. Middleton RWD, Frost RB: Percutaneous intramedullary rod interchange in osteogenesis imperfecta. *J Bone Joint Surg (Br)* 1987; 69:429.
87. Benson DR, Newman DC: The spine and surgical treatment in osteogenesis imperfecta. *Clin Orthop Rel Res* 1981; 159:147.
88. Yong-Hing K, MacEwen GD: Scoliosis associated with osteogenesis imperfecta. *J Bone Joint Surg (Br)* 1982; 64:36.
89. Cristofaro RL, Hoek KJ, Bonnett CA, et al: Operative treatment of spine deformity in osteogenesis imperfecta. *Clin Orthop Rel Res* 1978; 139:40.
90. Pauli RM, Gilbert EF: Upper cervical cord compressions as cause of death in osteogenesis imperfecta type II. *J Peds* 1985; 108:579.
91. Pozo JL, Crockard HA, Ransford AO: Basilar impression in osteogenesis imperfecta. *J Bone Joint Surg (Br)* 1984; 66:233.
92. Tsipouras P, Barabas G, Matthews WS: Neurologic correlates of osteogenesis imperfecta. *Arch Neurol* 1986; 43:150.
93. Lanting PJH, Borsboom PCF, te Meerman GT, et al: Decreased scattering coefficient of blue sclerae. *Clin Genet* 1985; 27:187.
94. Chan CC, Greene WR, de la Cruz ZC, et al: Ocular findings in osteogenesis imperfecta congenita. *Arch Ophthalmol* 1982; 100:1459.
95. Kaiser-Kupfer MI, McCain L, Shapiro JR, et al: Low ocular rigidity in patients with osteogenesis imperfecta. *Invest Ophthalmol Visual Sci* 1981; 20:807.
96. Kaiser-Kupfer MI, Podgor MJ, McCain L, et al: Correlation of ocular rigidity and blue sclerae in osteogenesis imperfecta. *Trans Ophthalmol Soc UK* 1984; 104:191.
97. Pederson V: Hearing loss in patients with osteogenesis imperfecta. *Scand Audio* 1984; 13:67.
98. Riedner ED, Levin LS, Holliday MJ: Hearing patterns in dominant osteogenesis imperfecta. *Arch Otolaryngol* 1980; 106:737.
99. Shapiro JR, Pikus A, Weiss G, Rowe DW: Hearing and middle ear function in osteogenesis imperfecta. *JAMA* 1982; 247:2120.
100. Brosnan M, Burns H, Jahn AF, et al: Surgery and histopathology of the stapes in osteogenesis imperfecta tarda. *Arch Otolaryngol* 1977; 103:294.
101. Hall JG, Rohrt T: The stapes in osteogenesis imperfecta. *Acta Otolaryngol* 1968; 65:345.
102. Pederson V, Sogaard H, Elbrond O: Histological investigation of skin biopsies in otosclerosis and osteogenesis imperfecta. *Arch Otorhinolaryngol* 1984; 240:1.
103. Pederson V, Elbrond O: Stapedectomy in osteogenesis imperfecta. *ORL* 1983; 45:330.
104. Shea JJ, Postma DS: Findings and long-term surgical results in the hearing loss of osteogenesis imperfecta. *Arch Otolaryngol* 1982; 108:467.
105. Armstrong BW: Stapes surgery in patients with osteogenesis imperfecta. *Ann Otorhinolaryngol* 1984; 93:634.
106. Levin LS: The dentition in osteogenesis imperfecta syndromes. *Clin Orthop Rel Res* 1981; 159:64.
107. Sunderland EP, Smith CJ: The teeth in osteogenesis and dentinogenesis imperfecta. *Br Dent J* 1980; 149:287.
108. Schwartz S, Tsipouras P: Oral finding in osteogenesis imperfecta. *Oral Surg* 1984; 57:161.
109. Levin LS, Brady JM, Melnick M: Scanning electron microscopy of teeth in dominant osteogenesis imperfecta. *Am J Med Genet* 1980; 5:189.

110. Becher J, Schuppan D, Benzan H, et al: Immunohistochemical distribution of collagen types IV, V, and VI and of pro-collagen type I and III in human alveolar bone and dentin. *J Histochem Cytochem* 1986; 34:1417.
111. Stenvik A, Larheim R, Stochaug K: Incisor and jaw relationship in 27 persons with osteogenesis imperfecta. *Scand J Dent Res* 1985; 93:56.
112. Levin LS, Wright JM, Byrd DL, et al: Osteogenesis imperfecta with unusual skeletal lesions: Report of three families. *Am J Med Genet* 1985; 21:257.
113. Pasquali-Ronchetti I, Quaglino D, Baccarani-Contri M, et al: Aortic elastin abnormalities in osteogenesis imperfecta type II. *Col Rel Res* 1986; 6:409.
114. Kalath S, Tsipouras P, Silver FH: Increased aortic root stiffness associated with osteogenesis imperfecta. *Ann Biomed Engin* 1987; 15:91.
115. Evensen SA, Myhre L, Stormorken H: Hemostatic studies in osteogenesis imperfecta. *Scand J Haematol* 1984; 33:177.

Measurements of Blood Flow

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Measurement of blood flow from the heart (cardiac output) or to segments of the circulatory system (regional or organ flow) may be approached by a variety of methods. With the possible exception of vessel transection and blood collection in a graduated cylinder (Fig 1), each method relies on the application of physiologic principle to biologic systems. Attendant to that application are potential sources of error that, together with biologic variability, make precise and reliable flow quantification problematic in some circumstances. Errors vary between methods and sites of measurement. Accordingly, the accuracy of measurements requires both a knowledge of basic principles and use of a technique appropriate to the clinical or experimental circumstance.

We review in detail measurement methods based on a number of general principles: conservation of matter (the Fick principle, dye dilution), radionuclide-labeled microsphere dispersion, and ultrasonic frequency shifts (Doppler).

Section I covers the Fick principle, residue detection, and microsphere applications.

Section II covers the indicator dilution method of flow measurement and its application to quantification of transit time, volume of distribution, and extravascular lung water.

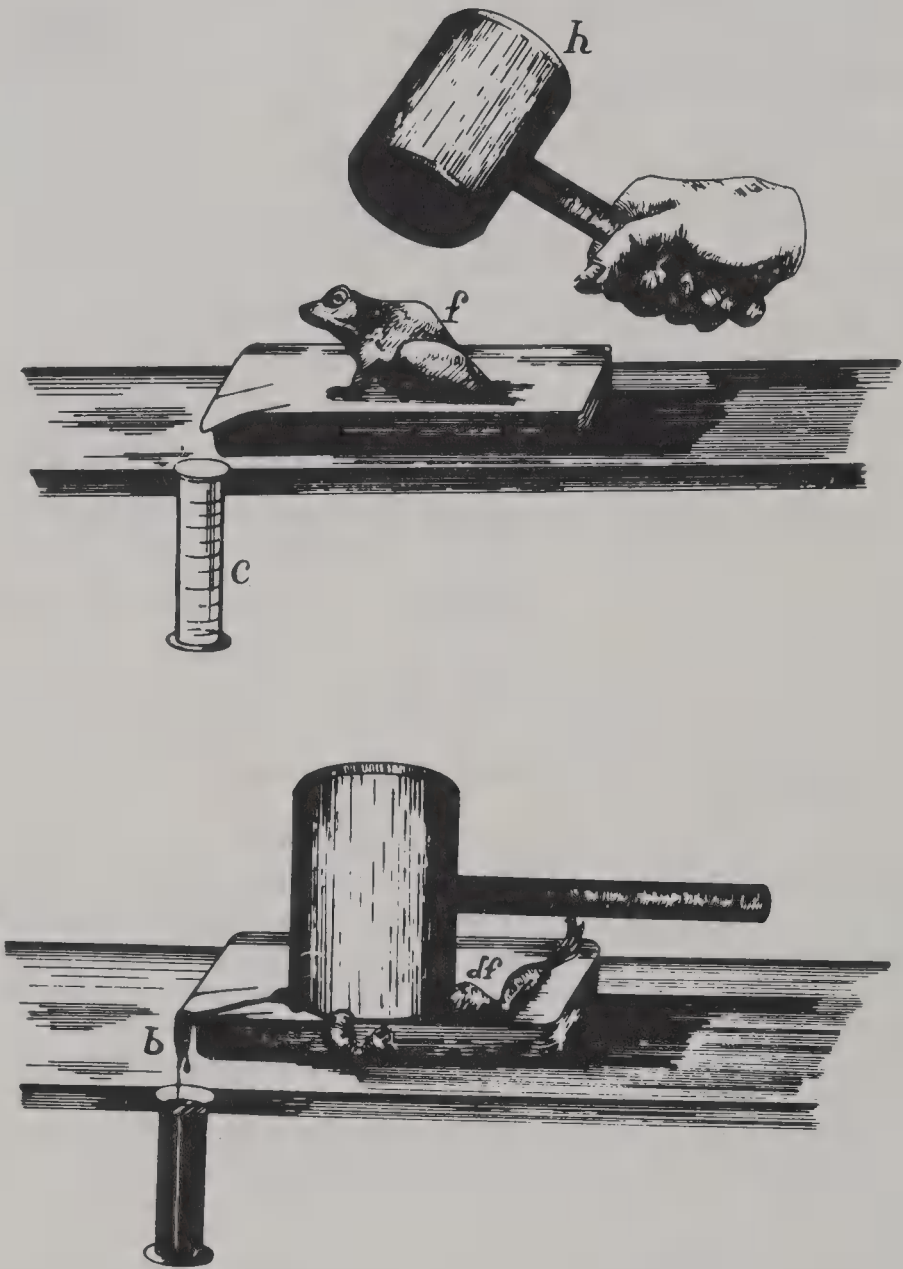


FIG 1.

Starling's method of determining the blood volume of a frog. The simplest and most direct method of flow measurement. Note the spontaneous sympathetic blockage that occurs concomitantly with the extraction of blood. (Courtesy of David Clive, M.D., Yale University.)

Section III reviews flow measurement by ultrasound methods.

In each section, principles, application, and sources of error will be discussed.

Blood Flow Measurement*

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Fick Principle

The Fick principle is based on the law of conservation of matter. With appropriate indicators the method can be used to measure cardiac output, flow to various organs, and even flow to small regions within organs. Many of the applications of the Fick method involve measurement of arterial and venous concentrations of the indicator although sometimes, by judicious choice of indicator, only one of these concentrations needs to be measured. Other applications of the method measure the amount of indicator left in the tissue after its injection (residue detection); this technique is used either for measuring flows to small regions in the tissue or else for external measurement of a radioactive indicator in intact animals and humans.

Constant Concentration Methods

Consider an organ with a constant flow of blood at F ml/minute into its artery and out through its vein. Suppose, too, that there is a constant arteriovenous difference of an indicator, with arterial and venous concentrations respectively C_a and $C_{\bar{v}}$ units/ml (\bar{v} indicates the mixed venous concentration for that organ); examples might be oxygen or lactic acid across various vascular beds. Because concentration (C) is quantity (Q) divided by volume (V), then $Q = CV$. Therefore, the quantity of indicator brought into the organ each minute (\dot{Q}_a) is $C_a \dot{V}$, where \dot{V} is the volume entering the organ each minute; because $\dot{V} = F$ ml/minute, then $\dot{Q}_a = FC_a$, and similarly $\dot{Q}_{\bar{v}} = FC_{\bar{v}}$. Thus, each minute \dot{Q}_a units of indicator enter and $\dot{Q}_{\bar{v}}$ units of it leave the organ. $\dot{Q}_{\bar{v}}$ will be less than \dot{Q}_a if indicator is metabolized in tissue, excreted from it or stored in it; on the other hand, $\dot{Q}_{\bar{v}}$ will exceed \dot{Q}_a if indicator is made in tissue or diffuses into it from somewhere other than blood. To measure flow, an indicator is chosen so that only one of these factors causes an arteriovenous difference; the indicator can be endogenous, as is oxygen, or exogenous, as is indocyanine green or nitrous oxide. If we can measure the quantity of indicator that causes the difference between the quantities entering and leaving the system (\dot{Q}_x), then we can readily calculate flow:

$$\dot{Q}_x = \dot{Q}_a - \dot{Q}_{\bar{v}} = F(C_a - C_{\bar{v}}) \quad (1)$$

*This work was supported in part by Program Project Grant HL25847.

so that

$$F = \frac{\dot{Q}_x}{C_a - C_v} \quad (2)$$

The best known example of this steady concentration Fick method is the conventional method of measuring cardiac output. Here \dot{Q}_x is the quantity of oxygen ($\dot{V}O_2$) used each minute by the body and also, in the steady state, taken into the body through the lungs. For pulmonary flow (\dot{Q}_p in L/minute), the relevant oxygen concentrations are in pulmonary veins (C_{pv}) and pulmonary arteries (C_{pa}), both in ml/L. For systemic flow (\dot{Q}_s in L/minute), the relevant oxygen concentrations are in aorta (C_{ao}) and mixed venous blood (C_{mv}), also in ml/L. Therefore,

$$\dot{Q}_p = \frac{\dot{V}O_2}{(C_{pv} - C_{pa}) O_2}$$

and

$$\dot{Q}_s = \frac{\dot{V}O_2}{(C_{ao} - C_{mv}) O_2}$$

Because normally $C_{pv} = C_{ao}$ and $C_{pa} = C_{mv}$, pulmonary and systemic flows are the same (Table 1, column A).

This method can be used to measure flow to individual organs if there is an indicator that will allow an estimate of \dot{Q}_x . Thus, to measure splanchnic blood flow, indocyanine green dye is infused intravenously at a known rate and serial samples are drawn from an artery and a hepatic vein to determine the concentrations of the dye in blood.¹ When the arterial concentration of indocyanine green is constant, then the excretion of indocyanine green by the liver just matches the quantity infused, which is therefore \dot{Q}_x ; with this value and the arteriovenous concentration difference of dye across the liver, the flow can be calculated. Similarly, renal blood flow can be measured by infusing at a steady rate an indicator, paraamino hippuric acid (PAH), that is excreted by the kidneys. Once the arterial concentration of PAH is constant, then renal flow can be calculated from the known amount of PAH infused each minute (\dot{Q}_x) and the arteriovenous difference of PAH concentrations across the kidney. In practice, the renal vein concentration of PAH is not usually measured because it is known to be about 5% of the arterial concentration; if PAH extraction is suspected to be other than 95%, then PAH concentration must be measured in the renal vein.

Problems of the Constant Concentration Fick Method

Circulatory Shunts

If there is a left-to-right shunt, C_{pv} and C_{ao} will be the same, but C_{pa} will be higher than C_{mv} (see Table 1, column B). Then \dot{Q}_p exceeds \dot{Q}_s so

TABLE 1.
Sample Calculations of Pulmonary and Systemic Flows and Shunts*

	A. No Shunt	B. L→R Shunt	C. R→L Shunt	D. Bidirectional Shunt
$\dot{V}O_2$ (ml/min)	240	240	240	240
Cpv (ml/L)	200	200	200	200
Cao (ml/L)	200	200	180	180
Cpa (ml/L)	160	180	120	160
Cmv (ml/L)	160	160	120	120
\dot{Q}_p (L/min)	6.0	12.0	3.0	6.0
\dot{Q}_s (L/min)	6.0	6.0	4.0	4.0
\dot{Q}_{ep} (L/min)	6.0	6.0	3.0	3.0
\dot{Q}_{lr} (L/min)	...	6.0	...	3.0
\dot{Q}_{rl} (L/min)	1.0	1.0

*Although the symbol for flow is usually F , the tradition of using \dot{Q}_p and \dot{Q}_s for pulmonary and systemic flow will be retained here.

that the left-to-right shunt (\dot{Q}_{lr}) is the difference, 6 L/minute. If there is a right-to-left shunt (\dot{Q}_{rl}), then $C_{pa} = C_{mv}$ but C_{pv} is greater than C_{ao} . Therefore, \dot{Q}_s exceeds \dot{Q}_p , so that the right-to-left shunt is the difference, 1 L/minute (see Table 1, column C).

If there are both left-to-right and right-to-left shunts, the flows are best calculated by using the concept of effective pulmonary blood flow (\dot{Q}_{ep}). This is defined as the quantity of systemic venous blood that is oxygenated in the lungs, and it is calculated from $\dot{Q}_{ep} = \dot{V}O_2 / (C_{pv} - C_{mv})O_2$. (If there are no shunts, this is the same as either systemic or pulmonary flow.) If \dot{Q}_p is greater than \dot{Q}_{ep} , then there is a left-to-right shunt of amount $\dot{Q}_p - \dot{Q}_{ep}$. Similarly, if \dot{Q}_s exceeds \dot{Q}_{ep} , then there must be a right-to-left shunt of amount $\dot{Q}_s - \dot{Q}_{ep}$ (see Table 1, column D).

Although the principles of measuring these flows and shunts are straightforward, there are many sources of error. First, the results are only as accurate as are the measurements of oxygen consumption and oxygen contents, so that there must be accurate methods as well as an experienced person making the measurements. Second, if there is a very high pulmonary blood flow, then the arteriovenous difference of oxygen across the lungs is small and slight errors in determining the oxygen contents can cause large errors in the estimate of pulmonary blood flow. For example, large left-to-right shunts may have saturations in pulmonary venous and pulmonary arterial blood of 95% and 90%, respectively. A 1% error in measuring saturation is quite low, but if this error is in opposite directions in the two blood samples, it could lead to a difference of oxygen saturation across the lung that, instead of 5%, is either 3% or 7%. If the oxygen carrying capacity of blood is 200 ml/L, then the arteriovenous differences of oxygen concentration across the lung should be 5% of 200 = 10 ml/L, but could vary because of error in determining saturation from 6 to 14 ml/L. If $\dot{V}O_2 = 240$ ml/min, then \dot{Q}_p should be $240/10 = 24$ L/minute, but could vary from $240/6 = 40$ L/minute to $240/14 = 17.1$ L/minute. In general, when pulmonary flows are very high, it is not possible to be sure of the exact values; flows of 40 L/minute at one time and 25 L/minute later could not with certainty be taken to differ.

There are major problems relating to how well the blood sample represents the blood that is required. In transposition of the great arteries, for example, pulmonary blood flow is calculated from pulmonary venous and pulmonary arterial samples, but the latter do not include the bronchial arterial blood of low saturation that enters the distal pulmonary arterial branches; as a result, the true oxygen concentration of blood entering the alveoli to pick up oxygen is overestimated. Because of the small arteriovenous oxygen difference across the lung, even as little as 10% of bronchial blood entering the pulmonary circulation can produce a 50% error in calculated pulmonary blood flow.²

Obtaining well-mixed samples is difficult when there are shunts. Normally the best mixed venous blood is obtained from the pulmonary arteries, preferably in the larger branches to avoid the error from diffusion of

oxygen from alveoli through the walls of the smaller arteries. When there is a patent ductus arteriosus, however, there can be differences between the saturations of blood in right and left pulmonary arteries, with an average of 5% more in the left than the right pulmonary artery. Thus, it is best to calculate an average pulmonary arterial content using saturations from each artery, although because the relative flows in each artery are not known, a true weighted mean pulmonary arterial oxygen saturation cannot be obtained. It also is crucial to obtain the pulmonary arterial samples fairly far from the connection of the ductus arteriosus to the pulmonary artery or else what is measured is mainly undiluted arterial blood that gives a gross overestimate of the pulmonary blood flow. This problem also prevents accurate measurement of pulmonary blood flow in newborn infants, especially if premature, because when there is a ductus arteriosus, it may not be possible to pass the catheter into the distal pulmonary arteries.

If there is a ventricular septal defect, then the larger pulmonary arterial branches usually give a good estimate of mixed postshunt oxygen saturation, but there is some difficulty obtaining a mixed venous saturation. This cannot be derived from right ventricular blood because some oxygenated shunt blood will be included in the sample, and right atrial blood samples are not useful because many children with large ventricular septal defects have either tricuspid incompetence or else a left-to-right atrial shunt. Therefore, vena caval blood will have to be used, and because there are usually differences in saturation in the superior and inferior venae cavae, some average will have to be taken. This difficulty is worsened when there is a large left-to-right atrial shunt, both because right atrial blood cannot be used for a mixed venous value and because there often is regurgitation of right atrial blood into the cavae. Thus, sampling from the cavae near the right atrium may include some oxygenated shunt blood, and sampling blood in the cavae far from the heart tends to give values that reflect saturations in a local stream of blood and not a true mixed venous value. In fact, estimates of systemic flow in those with large atrial septal defects frequently are unreliable when calculated by this method.³

Unsteady States

Basic assumptions in equations 1 and 2 are that flows and concentrations are constant. This leads to difficulties in measurements of splanchnic and renal blood flow because it may take 20 or 30 minutes of infusion time to obtain a steady arterial concentration of indicator. Once the measurement has been made, any change in flow caused by drugs or exercise will require another fairly long time for equilibration to occur, so that it is not possible to obtain frequent estimates of blood flow; furthermore, the assumption of steady flow throughout the period of measurement is difficult to verify. This problem should be less serious for measurements of cardiac output because, as a rule, the samples are collected over 1 to 3 minutes. However, cardiac output can change rapidly with anxiety or movement, so that a true steady state may be difficult to obtain. Furthermore, pulmo-

nary and aortic blood flows are not steady, but change during the cardiac and respiratory cycles, as do venous oxygen concentrations. The changes in the cardiac cycle probably do not introduce inaccuracies, but the variations in pulmonary flow with respiration, especially during exercise, need to be considered further.⁴ The error introduced by flow variations is due to the fact that the blood samples are usually drawn at a steady rate and so give a *time-averaged* sample, whereas what is needed is a *volume-averaged* sample. The magnitude of this error clearly will depend on the actual flows and durations of inspiration and expiration concerned; the error probably is not large at rest, but could be large on exercise. Visscher and Johnson⁴ point out that errors up to 50% could occur if there were marked phasic changes of flow and concentrations. If either of these is constant, then changes in the other cause no error.

If cardiac output is measured during exercise, about 2 minutes of steady exercise are needed to achieve a relatively steady state.⁵

Inert Gas Methods for Cardiac Output

Carbon Dioxide

One of the major disadvantages of the Fick method for measuring cardiac output is that it requires inserting catheters and needles into the subject. Therefore, many attempts have been made to measure cardiac output by less invasive techniques, particularly with various gas methods. One approach that has been tried is to use carbon dioxide rather than oxygen as the indicator. Because carbon dioxide can be measured readily in collected expired air to give \dot{Q}_x , and because arterial carbon dioxide concentration can be equated with end tidal gas measurements, all that remains is to estimate pulmonary arterial (mixed venous) carbon dioxide concentration and then the Fick calculation can be done. One way of making this estimate is to have the subject rebreathe from a bag containing carbon dioxide at approximately the expected mixed venous concentration. As the subject breathes in and out, inspired and expired carbon dioxide concentrations are monitored. When carbon dioxide concentration does not change during inspiration and expiration, then the concentration must be the same as that in the blood entering the pulmonary capillaries and can be used in the Fick equation. A simple and reliable method for determining mixed venous carbon dioxide concentrations has been reported.⁶ Unfortunately, this appealing method has many problems.⁷ The difference in tensions and concentrations of carbon dioxide between pulmonary artery and pulmonary veins is small, so that error in estimating those values will produce a large error in the estimated flows. In addition, the transient increase in carbon dioxide concentration causes difficulties related both to the solubility of

carbon dioxide in lung tissue and to the changes of hemoglobin binding to carbon dioxide.

Godfrey and his colleagues⁸ recently have revived the carbon dioxide method for measuring cardiac output in children during exercise and have obtained good correlations between outputs measured by this method and by an indicator dilution method. There are two advantages of the method: (1) it will not be affected by regions with low ventilation-perfusion ratios in which alveolar and arterial carbon dioxide tensions will be in equilibrium and (2) the accuracy of the method improves with exercise because the arteriovenous difference for carbon dioxide increases at each level of exercise. The method can be used in children over 5 or 6 years old.

Nitrous Oxide

It is possible to use foreign gases that dissolve in the blood perfusing the alveoli. Acetylene once was used for this purpose, and some of the earliest data on cardiac output came from its use; it recently has been reintroduced.⁹ However, nitrous oxide, introduced by Krogh and reintroduced by Lee and DuBois in 1955,¹⁰ is used more often. If (N_2O) in the alveoli has a fractional concentration of f_A , then it has a partial pressure of $f_A (P_b - 47)$ torr, where P_b is the barometric pressure and 47 is the correction for water vapor at 37 C. Because of its solubility in blood and its rapid diffusion across the capillary membranes, nitrous oxide will have the same partial pressure in the alveoli and the pulmonary capillary blood; thus, the quantity dissolved per ml of blood will be

$$\lambda f_A \frac{(P_b - 47)}{760} \text{ ml}$$

where λ is the solubility coefficient of nitrous oxide in ml per ml blood at 760 mm Hg. If the flow through the pulmonary capillaries is \dot{Q}_c (ml/minute), then the amount of nitrous oxide removed per minute by the blood is

$$\lambda f_A \frac{(P_b - 47)}{760} \dot{Q}_c$$

so that flow can be calculated as

$$\dot{Q}_c = \frac{\dot{V}_{N_2O}}{\lambda f_A \frac{(P_b - 47)}{760}} \quad (3)$$

Here the denominator is equivalent to C_a in equation 2; $C_{\bar{v}}$ is zero prior to recirculation. Lee and DuBois¹⁰ introduced a method for instantaneous pulmonary capillary flow measurement in which the subject sat in a body

plethysmograph, breathed in a low concentration of nitrous oxide, held the breath for 10 to 15 seconds with an open glottis, and then breathed out. The value f_A was measured in expired gas by an infrared analyzer, and the volume of nitrous oxide removed (VN_2O ml) was determined by the change of pressure within the plethysmograph. Wasserman and Comroe¹¹ eliminated the plethysmograph by measuring the volume of nitrous oxide removed from a sensitive Krogh spirometer. Bosman et al.¹² retained the plethysmograph, but allowed its pressure to be maintained by inflow of air so that the record of air inflow into the plethysmograph was the same as that of nitrous oxide uptake by the lungs. All these methods allow measurement of phasic as well as mean nitrous oxide uptake; therefore, variations in pulmonary capillary flow throughout the cardiac cycle can be measured. The various assumptions and complexities of this basically simple method are well discussed in the review by Butler.⁷

The drawbacks to these methods are that breath-holding may be difficult for small children or sick people, breath-holding itself may alter the circulation, studies must be done at rest, results are inaccurate if there is much uneven distribution of ventilation, the flow measured is only to ventilated alveoli, and measurements must be made before recirculation occurs so that they cannot be made when there are large left-to-right shunts. Becklake et al.¹³ introduced a variant of the method to allow nitrous oxide uptake to be measured during exercise. Ayotte et al.¹⁴ further modified the method by having the patient rebreathe from a bag containing nitrous oxide as well as nonabsorbable inert gases. The nonabsorbed gases were used to measure lung volume, and the amount of nitrous oxide taken up was calculated from the integrated difference between the initial and final nitrous oxide concentrations in the total volume of bag and lung. Both of these methods allow measurements to be made during exercise, the breath is not held, and uneven distribution of ventilation has much less effect on the accuracy of the results. However, the other problems remain.

Methods With Varying Vascular Concentrations

Basic Kety-Schmidt Method

If the concentrations of indicator in arterial and venous blood change with time, then equation 2 may be modified. In any small time interval (dt) the amount of indicator accumulating in tissue, for example is dQ_x and in this time interval flow is calculated as

$$Fdt = \frac{dQ_x}{C_a - C_v} \quad (4)$$

This can be rearranged to give

$$dQ_x/dt = F (C_a - C_v) \quad (5)$$

or

$$d\dot{Q}_x = Fdt (C_a - C_{\bar{v}}) \quad (6)$$

Therefore, over a period of u minutes, we can integrate both sides of equation 6 to give

$$\dot{Q}_x(u) = F \int_0^u (C_a - C_{\bar{v}})dt \quad \text{because } F \text{ is constant,}$$

which can be rearranged to give

$$F = \frac{\dot{Q}_x(u)}{\int_0^u (C_a - C_{\bar{v}})dt} \quad (7)$$

where $\dot{Q}_x(u)$ is the quantity of indicator accumulating in u minutes and $\int_0^u (C_a - C_{\bar{v}})dt$ is the integrated arteriovenous difference over that period.

Note that this method still requires flow to be constant.

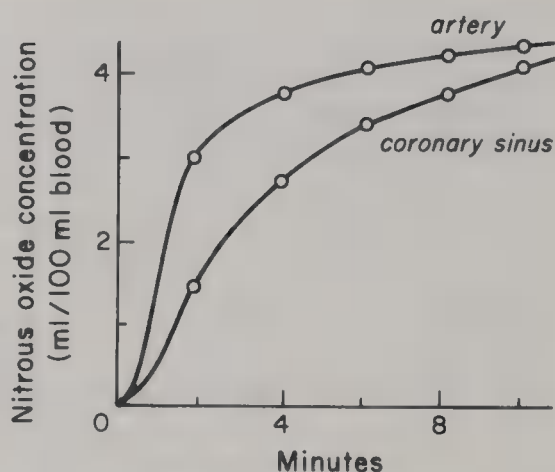
It is necessary to use this form of the Fick equation for organs like the brain or the heart because indicators of the type used for kidney or splanchnic bed are not available and thus there is no way of measuring \dot{Q}_x except to remove the organ. One way around the difficulty is to use inert indicators that are taken up by tissues so that sooner or later concentrations of indicator in blood and tissues achieve equilibrium. At equilibrium, the concentrations of indicator in blood or tissue are not necessarily the same; the ratio of these two concentrations is termed the tissue-blood partition coefficient (λ_{tb}) and is determined empirically. For example, for nitrous oxide and antipyrine the partition coefficient is about 1, whereas for xenon it is about 0.72. What this means is that at equilibrium the concentrations of nitrous oxide or antipyrine are similar in blood and tissue, but xenon concentration in tissues is 72% of that in blood.^{15, 16}

To measure coronary (or cerebral) blood flow with nitrous oxide, the patient or animal breathes a low concentration of nitrous oxide, and serial samples of arterial and coronary sinus (or jugular vein) blood are taken over 10 to 15 minutes.¹⁷ The plot of these concentrations against time is in Figure 2. The area between the two curves is $\int_0^u (C_a - C_{\bar{v}})dt$, the denominator of equation 7. The numerator cannot be obtained in absolute terms; therefore, another approach must be used. Consider a volume of tissue V_x and divide it into both sides of equation. Then

$$\frac{F}{V_x} = \frac{\dot{Q}_x(u)/V_x}{\int_0^u (C_a - C_{\bar{v}})dt} = \frac{C_x(u)}{\int_0^u (C_a - C_{\bar{v}})dt} \quad (8)$$

FIG 2.

Example of arterial and coronary sinus nitrous oxide saturation curves for measuring coronary blood flow. Circles indicate when samples were taken. (From McGregor M: Significance of myocardial flow measurement in the evaluation of the coronary patient, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 287–296. Used by permission.)



Now at equilibrium C_x and C_v are related by $C_x = \lambda_{tb}C_v$, so that at equilibrium the equation becomes

$$\frac{F}{V_x} = \frac{\lambda_{tb}C\bar{v}(u)}{\int_0^u (C_a - C\bar{v})dt} \quad (9)$$

Therefore, knowing λ_{tb} , $C\bar{v}$ at equilibrium, and the area between the arterial and coronary sinus (or jugular vein) curves, we can calculate the coronary (or cerebral) blood flow per unit volume of tissue. If blood concentrations of nitrous oxide are measured in ml/100 ml, then we obtain flow per 100 ml of tissue—either left ventricle or cerebrum. Because tissue specific gravity is about 1.06, flow per 100 gm of tissue will be the above measured flow divided by 1.06.

The nitrous oxide technique is done most often by allowing the patient or animal to breathe low concentrations of nitrous oxide for 10 to 20 minutes so that tissue-blood equilibrium is reached. Then the nitrous oxide breathing mixture is removed, and arterial and coronary sinus (or jugular vein) blood is sampled at intervals for 10 to 15 minutes as nitrous oxide is washed out. The use of desaturation rather than saturation methods allows more stability (because mouthpieces are not needed for the former), avoids error due to leakage at the mouthpiece, and allows measurement of small terminal arteriovenous differences to be made when each is small rather than when each is big.^{18, 19}

Even with this method there are major problems. First, it is essential to ensure that the venous sample is not contaminated with blood from some other region; thus, coronary sinus blood must be free of right atrial contamination, and jugular vein blood must not be mixed with extracerebral venous blood. Second, there is error due to inhomogeneous flows. If some regions of the myocardium have much reduced flows, then it will take much longer to saturate or desaturate them than regions with higher flows.

This is a source of considerable error, and Klocke and Wittenberg¹⁹ and Klocke et al.²⁰ have pointed out that it may be impossible to achieve a uniform concentration of nitrous oxide in heterogeneously perfused regions while arterial concentrations are still changing. This difficulty applies equally to saturation and desaturation curves. For example, during wash-out, regions with low flows retain nitrous oxide longer than do others with normal flows, and there will be a very long tail to the coronary sinus curves, so that either the sampling is completed before all the heart is cleared of nitrous oxide or else the very low terminal levels cannot be measured with accuracy. As a result, because the value for the integrated arteriovenous difference tends to be too small, flow will be overestimated; errors of as much as 100% can occur.^{20, 21} These problems are not abolished by using other indicators, although some, such as argon and helium, may be easier to measure in very low concentrations^{20, 22}; the difficulties due to regional variation in perfusion remain. Finally, even if the flow could be measured accurately, it is doubtful whether there is useful physiologic information to be gained by measuring an average flow to an organ in which there are large regional differences. The problem for the heart has been highlighted by Hoffman and Buckberg.²³

Residue Detection

The inert gas method requires the insertion of catheters into arteries and veins and also requires removal of blood and often laborious analysis of the quantities of gas in the blood samples. To overcome some of these difficulties, radioactive xenon was introduced as a technique for measuring coronary blood flow.²⁴ Its use can be understood by returning to equation 5:

$$\frac{d\dot{Q}_x}{dt} = F(C_a - C_v) \quad (5)$$

Now if an inert gas, for example xenon, is injected into the coronary artery, it is distributed to the tissues, equilibrates with them, and then is washed out by the incoming xenon-free arterial blood. Therefore, C_a is zero and the equation becomes

$$d\dot{Q}_x/dt = -FC_v = -\frac{\dot{Q}_x F}{\lambda V_x}, \text{ because } C_v = \frac{\dot{Q}_x}{\lambda V_x} \quad (10)$$

This is a first order differential equation that has as its solution

$$\dot{Q}_x(u) = \dot{Q}_x(o)e^{-ku} \quad (11)$$

Here $\dot{Q}_x(u)$ and $\dot{Q}_x(o)$ are the quantities of indicator at times u and zero, respectively, and the rate constant $k = F/\lambda V_x$, so that $F = k\lambda V_x$. If the myocardial specific gravity is 1.06, then $V_x = W_x/1.06$ where W_x is the weight of tissue being examined. Therefore, $F = k\lambda W_x/1.06$. Because W_x normally is not known, it is set at 100 gm, and thus flow/minute/100 gm

is measured from $100 \times k\lambda/1.06$. The rate constant k is determined from the exponential decay of the xenon washout curve; if this is plotted on semilogarithmic paper, then k is $\log_e 2/t^{1/2}$ (or $0.693/t^{1/2}$), where $t^{1/2}$ is the time taken for the count rate to fall to one half of its peak value.²⁴

Even though the xenon must be injected into the coronary artery via a catheter, the washout curve can be obtained directly from a counter and ratemeter over the chest. Unfortunately, the washout curve is markedly affected by regional variations of perfusion because if there is not a single exponential washout, a true rate constant cannot be obtained.^{25, 26} If there are two compartments, one with high and one with low flow, then in theory, the washout curve can be broken down into two slopes so that the volume and flow rate of each compartment can be determined. However, even normal hearts appear not to yield monoexponential slopes regularly,^{76,77} and a careful study of xenon washout curves in isolated, perfused dog hearts with known flows has revealed unexpectedly large errors with use of either one or two compartment analyses. Second, there may well be more than two compartments, especially in diseased hearts. Third, in order to define the presence of two or more slopes, it is necessary to record to very low count rates that may be impossible to separate from background radiation. The error that comes from ignoring a compartment with a slow flow may be large; in general, the false assumption of a monoexponential washout tends to give too low a calculated flow, but this is not always true nor is the error constant (Fig 3).²⁷ Finally, xenon has high solubility in fat and so tends to accumulate in poorly perfused cardiac fatty tissue from which it may later recirculate.^{28, 29}

One way of reducing these errors is to record by multiple collimators the washouts in numerous portions of the heart.^{30, 31} This reduces the heterogeneity that may occur in adjacent regions, but does not guarantee monoexponential flows, both because there can be much variability in the small regions under a single counter³²⁻³⁴ and because in disease there is often reduced subendocardial flow. Analyses of coronary flow by washout curves is therefore of unknown accuracy, especially in those disease states where it might be most wanted.

The technique of residue detection most often is done with radioactive indicators; thus, external counting may be used in intact animals. However, residue detection can be done after injection of radioactive xenon into the myocardium at the time of surgery³⁵ or by measuring hydrogen washout with platinum electrodes inserted into the myocardium after the myocardium has been saturated with hydrogen taken in by the lungs.^{36, 37} These methods do allow measurement at a single point so that inhomogeneity is not a problem, but they are naturally restricted in application, do not give integrated values for the whole heart, and can cause local damage that alters flows.

Recently, ^{81m}Kr has been introduced to overcome some of the problems inherent in inert gas techniques, in particular the need for a long steady state.³⁸ ^{81m}Krypton is a radioactive gas with a half-life of 13 seconds; it can

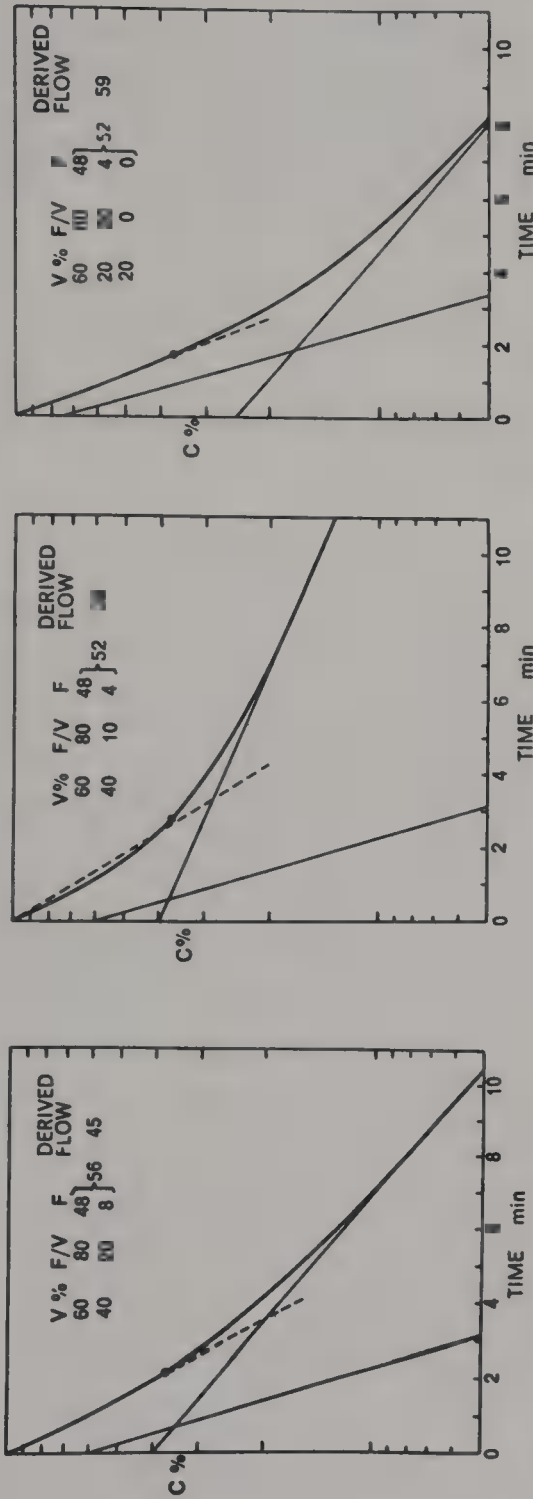


FIG 3.

Left, two compartments of the heart with different flows (F), volumes (V) and F/V ratios; even if there is good equilibration, fitting an exponential downslope too high on the curve underestimates the total flow. Middle, if flow to the slow compartment is reduced, fitting the exponential to the first 60% of the curve results in an even greater error. Right, if the slow compartment is affected by disease so that half of it retains its flow but the other half has no flow, now extrapolation after 60% of the curve causes an overestimate of total flow. (Redrawn from McGregor M: Significance of myocardial flow measurement in the evaluation of the coronary patient, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 287-296. Used by permission.)

be eluted continuously from an ^{81}Rb generator, dissolved in 5% dextrose saline, and infused at a constant rate into the aortic root. After the infusion is started, the concentration of the indicator in heart muscle will rise to a steady level that is well below the arterial concentration because some radioactivity is lost while the nuclide is travelling to the heart.³⁹ Any increase in blood flow to a region is followed rapidly by an increased radioactivity of the tissue, and a decrease in blood flow is followed rapidly by a decrease in local radioactivity.

It is possible to calculate flow per unit volume by modifying the Fick equation to take account of the radioactive decay of the $^{81\text{m}}\text{Kr}$.^{38, 39} The method is given in the appendix.

Flow to Very Small Regions

Inert Diffusible Indicators

With the exception of the multiple collimator method, none of the other techniques mentioned so far can measure flows to small regions within organs because it is difficult or impossible to isolate local venous drainages. This difficulty may be overcome by giving inert, diffusible, flow-limited indicators like xenon, argon, hydrogen, tritiated water, or antipyrine. These substances have all been shown to be flow limited but not diffusion limited; that is, they diffuse so rapidly into tissues that by the time blood has reached the venous end of the capillary, the concentrations of indicator in tissue and blood are in equilibrium. Therefore, if there is no diffusion barrier (and no arteriovenous shunting), the venous concentration can be estimated from the tissue concentration at any moment. After infusing the indicator for u seconds, the organ can be removed rapidly and cut into pieces that are analyzed for their indicator concentrations. Flow then is calculated in one of several ways.

If the indicator is infused so that there is a steady arterial concentration, the indicator passes from blood to tissue until the concentrations have equilibrated. Because tissue volume usually is much greater than blood volume, the tissues act as a sink to take up indicator. Therefore, the concentration of indicator in tissue and venous blood is very low at first. As more indicator reaches the tissue, it again equilibrates in tissue and blood. The quantity of indicator in the tissue rises, but its concentration in the tissue and venous blood remains low so that a lot of indicator enters in the first few seconds but very little leaves. Therefore, as a first approximation, the amount of indicator in a piece of tissue is proportional to the flow to that piece, which is $\dot{Q}x/\text{Ca}$. A piece of tissue having twice the flow of another piece will have twice the amount of indicator.

To be more accurate, consider what determines the diffusion of the indicator from blood to tissue. The amount moving from blood to tissue per

unit time depends on the area of the diffusing surface, the diffusivity of that indicator, and on the concentration gradient of indicator between tissue and blood. As tissue concentration rises the gradient from tissue to blood and hence the rate of indicator transfer fall, and the relationship is exponential. To see how to use this to measure regional flows, return to equation 5 and divide both sides by V_x to get:

$$\frac{d\dot{Q}_x}{V_x dt} = \frac{dC_x}{dt} = \frac{(C_a - C_{v_i})F}{V_x} \quad (16)$$

Here C_{v_i} is used in place of $C_{\bar{v}}$ because it refers to concentration in a small local vein, not to a mixed venous concentration. The corresponding tissue concentration is C_{x_i} . Now because

$$C_{x_i} = \lambda C_{v_i}$$

then,

$$\frac{dC_{x_i}}{dt} = \frac{(C_a - C_{x_i}/\lambda)F}{V_x} = \frac{(\lambda C_a - C_{x_i})F}{\lambda V_x} = k(\lambda C_a - C_{x_i}) \quad (17)$$

because

$$k = F/\lambda V_x$$

This equation may be integrated and solved for C_x at any time u for any of the three usual experimental conditions (see appendix).⁴⁰

These methods for measuring flow to small regions can eliminate the problems due to regional variations in flow, but still have errors. The partition coefficient of the indicators may vary with hematocrit and must be known, and there can be errors due to the amount of indicator in the arterial blood of the region at the time of analysis.⁴¹ Furthermore, at low flows, diffusional shunting of indicator from small arteries to their accompanying veins may occur. Nevertheless, these methods provide what is probably the best estimate of regional flows that can be obtained today.

A variation on this method recently has been introduced.⁴² When desmethylinipramine is infused, it is tightly bound to tissues; almost all of it is cleared in a single circulation through an organ. Therefore, the amount of this agent in a piece of tissue is proportional to the flow to that piece of tissue. The agent can be linked to a radioactive tracer so that it can easily be quantitated. The organ or organs of interest are removed after the infusion and cut up into pieces; if the specific activity of the desmethylinipramine in blood and the cardiac output are known, then the regional flows can be calculated.

Diffusible Cationic Indicators

Sapirstein^{43, 44} observed that for 30 to 90 seconds after infusing ⁴³K or ⁸⁶Rb into rats, the relative distribution of these isotopes remained constant

in all organs except the brain, which they enter poorly. Subsequently, ^{201}Tl was introduced so that it could be detected by scanning the heart with an external gamma camera. The three cations enter the large potassium sink in the cells, and for 90 seconds very little recirculates. Thus, the proportion of isotope in a region is similar to the population of the cardiac output that goes to that region.

This technique, the first to give information about relative flows to different parts of the heart, has many problems. To avoid redistribution of isotope from diffusion or recirculation, the animal must be killed and the organs removed within 90 seconds of the injection of the isotope. This means that serial measurements of blood flow are limited to a maximum of three measurements at about 30-second intervals. Second, the extraction ratio of these cations varies with flow, being reduced at higher flows. In addition, the three cations are not taken up by passive diffusion, but enter cells by active uptake; as a result, ischemia alters the kinetics of isotope accumulation in the damaged region.^{45, 46} These isotopes therefore are not used for accurate quantitation, although ^{201}Tl still is in common use clinically to image ischemic regions in which little of the isotope is taken up.

Positron Emission Tomography

An extension of the inert gas method that is capable of determining regional flow/volume ratios is obtained when positron emitting isotopes are used. Examples of such emitters are ^{11}C , ^{15}O , ^{82}Rb , and ^{13}N . Sometimes the nuclides are incorporated into an appropriate molecule that can be used to study metabolism. The heart is then scanned by an array of detectors that can register coincidence counts, and computerized tomographic techniques are used to localize the regions counted. These techniques have a resolution of 1 to 2 sq cm that is independent of depth, can correct for photon attenuation, and with corrections for image size can estimate local concentrations of indicator accurately.^{47, 48}

The indicator can be injected intravenously, into the left atrium, or even the coronary artery. If judiciously chosen, it accumulates in heart muscle in proportion to local flow rates and decreases rapidly in the circulation, thus minimizing recirculation; ^{13}N -ammonia fulfills some of these requirements.⁴⁹ To convert relative flows into absolute flows, the arterial admittance function is determined by sampling arterial blood⁵⁰ or by counting a sample volume in the left ventricular cavity. Positron emission tomography is invasive and has relatively poor time resolution, and the available indicators are not ideal. Nevertheless, it is one of the few methods that can measure flows to different regions within the heart, so that gross inhomogeneities of flow do not produce the inaccuracies that bedevil other clearance methods.

Recently, the group at Hammersmith Hospital in London has exploited

the use of $C^{15}O_2$ for measuring myocardial and cerebral blood flow.^{39, 51, 52} ^{15}O -labeled carbon dioxide is inhaled continuously. In the lung, there is a rapid transfer of ^{15}O to lung water, thus achieving a continuous arterial infusion of $H_2^{15}O$. Then the heart is imaged by a suitable positron emission tomograph, making use of equation 15 to calculate regional flows. With a $t_{1/2}$ of 123 seconds, this method yields good steady state measures of flow, but requires expensive equipment and a cyclotron on site.

Computerized Tomographic Angiography, Videodensitometry

If angiographic contrast medium is injected into an artery, a concentration-time curve can be determined at two sites on the artery. From these curves, the mean transit time between the sites can be determined, and the product of this and the measured cross sectional area of the artery estimates flow. Initially concentrations were determined by videodensitometry.^{53, 54} However, the introduction of computerized tomographic systems with scan times of 35 to 50 msec⁵⁵ will allow these density measurements to be made over coronary arteries or in different myocardial regions. Relative myocardial flows might well be measurable by these methods.

Radioactive Microsphere Methods

These are indicators that do not recirculate because they lodge in the capillary beds. The microspheres are made of polymerized plastic. The nuclide, which can be one of many varieties, is incorporated in the substance of the microsphere and does not leach out. These microspheres are virtually indestructible, and it is possible to give nine or more different sets of microspheres, each labeled with a different nuclide, and separate out their individual contributions later.⁵⁶ The measurement is made by injecting a known number of microspheres into the left atrium; the number is determined from their radioactivity and the known counts per microsphere. The microspheres mix well with the blood and then are distributed to each organ and to regions within organs in proportion to their blood flow during the time that the microspheres are being distributed.⁵⁷ To measure absolute flows, an arterial reference sample is drawn from any artery at a fixed rate. As far as the microspheres are concerned, the flow of blood from artery to the collecting vial is just like the flow into any other organ; thus, the vial receives a number of microspheres proportionate to its blood flow.⁵⁸ If the flow in the vial is Fr ml/minute and the number of counts in the vial is Cr /minute, then the flow to any piece of tissue (Ft) is calculated from the counts of nuclide in the tissue (Ct) as $Ft = Fr \times Ct / Cr$. Similarly, cardiac output (CO) may be calculated as $CO = Fr \times Ci / Cr$, where Ci is the total number of injected counts (Archie et al, 1973).⁵⁹ It also is possible to determine cardiac output repeatedly by injecting known amounts of radio-

active microspheres intravenously and withdrawing samples from the pulmonary artery.⁶⁰ In effect, this is like a continuous infusion in which flow is determined by dividing the amount injected by the concentration at equilibrium. The method has been validated in many studies. Cardiac output can be measured without bias and with a standard deviation of about 10%,⁵⁹ as can flow to numerous organs. To obtain this accuracy, certain requirements must be observed. It is best to inject the microspheres into the left atrium rather than the left ventricle to obtain adequate mixing. Then, a sufficient number of microspheres must be injected to ensure that at least 400 are present in any piece of tissue of interest and in the arterial reference sample. For example, if the left ventricle is the region of interest, then it should contain at least 400 microspheres, and if the posterior papillary muscle of the left ventricle is having its flow measured, then it should also have at least 400 microspheres. The method thus begins to break down if very tiny pieces of tissue are being examined (for example, portions of rat pituitary gland) because so many microspheres must be given to achieve accuracy that physiologic obstruction to flow could be produced. On the other hand, injection of 1 to 2 million microspheres into a 25-kg dog does not change the circulation and gives enough microspheres to measure flows to most regions with good accuracy. Note that it is number of spheres as well as numbers of counts that is needed for accuracy.^{61, 62}

The size of the microspheres also is important. Cardiac output can be measured with microspheres of any size from 9 to 100 μm diameter, but this may not be true of regional flows. In the heart, in which the problem has been investigated most intensively, the larger the microsphere, the more likely it is to move to the subendocardial muscle and to overestimate its flow.^{41, 63, 64} The probable reason for this is that, as the arteries pass into the deeper muscle, branching as they go, the larger microspheres that are more centrally placed in the vessels do not enter the lateral branches in proportion to their flows. Smaller microspheres from 9 to 15 μm in diameter probably do estimate regional flow in the heart within 10% of its true value in any layer or region. However, the smaller the microspheres, the greater the possibility that they will pass through the vascular bed being studied. In most organs, even 15- μm diameter spheres do not pass through in significant numbers,^{59, 65, 66} but this matter must be tested in each species, organ, and physiologic circumstance being studied. The method is reviewed in detail by Heymann et al.⁶⁷ and Hoffman et al.⁶⁸

Radioactive microspheres presently are the best way of measuring regional flows. Up to nine flows can be measured per animal, and with improved techniques, a dozen or more different nuclides could be used in each animal. There is no need to hurry to remove the organs to make the measurements that can be made in conscious animals. On the other hand, the animals must be killed and the organs removed; accordingly, for certain types of experiments and in humans, the method is not suitable. However, labeled albumin microspheres have been injected in humans to in-

dictate blood flow to various regions of the heart,⁶⁹ although, because of the geometric problems of detecting radioactivity from outside the body, the accuracy of the flow measurements is not known. Furthermore, the method as used in humans is at present unable to distinguish between subepicardial and subendocardial flows, and these may differ in any one region.

In humans, radionuclide-labeled albumin macroaggregates have been used to examine the distribution of blood flow to each lung after intravenous injection. Because the macroaggregates are trapped in the lung, their distribution to each lung and regions within the lungs indicates relative blood flows.⁷⁰⁻⁷² This method has been used to study various lung diseases, including pulmonary embolism, and also has indicated relative perfusion of each lung in congenital heart diseases with or without surgical shunts.⁷³ The method has been used recently in children with cyanotic congenital heart diseases to measure relative blood flows to lungs and body, because the macroaggregates that pass through the right to left shunt and so are not trapped in the lungs are trapped in the systemic vascular beds.⁷⁴

Appendix

^{81m}Kr Method for Measuring Flow Per Unit Volume

At equilibrium, the quantity of isotope entering the tissue and leaving it per unit time is in balance. The quantity entering is FCa ; the quantity leaving the tissue is the sum of the isotope that leaves in the blood ($FC\bar{v}$) plus the quantity that decays per unit time, and this is the product of the decay constant λ_d and the quantity of isotope in the tissue Qt . Therefore, at equilibrium,

$$FCa = FC\bar{v} + \lambda_d \dot{Q}t \quad (12)$$

However, because $\dot{Q}t = Ct \cdot Vt$ and $C\bar{v} = Ct/\lambda_{tb}$

$$FCa = FCt/\lambda_{tb} + \lambda_d \cdot Ct \cdot Vt \quad (13)$$

The partition coefficient (λ_{tb}) for ^{81m}Kr is 1, so that equation 13 simplifies to

$$FCa = FCt + \lambda_d Ct \cdot Vt \quad (14)$$

which can be rearranged to give

$$\frac{F}{Vt} = \frac{\lambda_d Ct}{(Ca - Ct)} = \frac{\lambda_d}{(Ca/Ct) - 1} \quad (15)$$

Because λ_d is a known physical constant of 3.2/minute, and Ca and Ct can be measured, F/Vt can be calculated.

This method gives the quickest response times of any of the inert gas techniques, but suffers from inability to localize differences of flows at different depths in the collimator field.

Equations for Inert, Diffusible Indicators

The basic equation is

$$\frac{dC_{x_i}}{dt} = k (\lambda C_a - C_{x_i}) \quad (17)$$

If C_a is constant, then

$$C_x(u) = \lambda C_a (1 - e^{-ku}) \quad (18)$$

If C_a is zero, then

$$C_x(u) = C_x(0)e^{-ku} \quad (19)$$

and if C_a is variable but is zero at time zero, then

$$C_x(u) = \lambda k e^{-ku} \int_0^u C_a e^{kt} dt \quad (20)$$

The form of the equation when C_a is zero is that used for the typical xenon washout. Equation 20 is the one most often encountered in practice, but it requires computer assistance for its solution.^{41, 75} Once k has been calculated, flow per unit volume or mass can be calculated.

References

1. Rowell LB: Measurement of hepatic-splanchnic blood flow in man by dye techniques, Bloomfield DA (ed): in *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 209-230.
2. Lakier JB, Stanger P, Heymann MA, et al: Early onset of pulmonary vascular obstruction in patients with aorto-pulmonary transposition and intact ventricular septum. *Circulation* 1975; 51:875-880.
3. Rudolph AM: *Congenital Diseases of the Heart*. Chicago, Year Book Medical Publishers, Inc, 1974, pp 120-130.
4. Visscher MB, Johnson JA: The Fick principle: Analysis of potential errors in its conventional application. *J Appl Physiol* 1953; 5:635-638.
5. Wade OL, Bishop JM: *Cardiac Output and Regional Blood Flow*. Oxford, Blackwell Scientific Publications, 1962.
6. Al-Dulymi R, Hainsworth R: A new open-circuit method for estimating carbon dioxide tension in mixed venous blood. *Clin Sci Molec Med* 1977; 52:377-382.
7. Butler J: Measurement of cardiac output using soluble gases, in Fenn WO, Rahn H (eds): *Handbook of Physiology*. Section 3; *Respiration*. Washington, DC, American Physiological Society, 1965, vol 2, pp 1489-1503.
8. Godfrey S: *Exercise Testing in Children*. Philadelphia, WB Saunders Co, 1974.

9. Severinghaus JW, Ozanne GW, Louderbough HC, et al: Lung water and cardiac output determination by noninvasive trace gas analysis of dual breath holds in a single breath. *Prog Resp Res* 1979; 11:280-296.
10. Lee G de J, DuBois AB: Pulmonary capillary blood flow in man. *J Clin Invest* 1955; 34:1380-1390.
11. Wasserman K, Comroe JH Jr: A method for estimating instantaneous pulmonary capillary blood flow in man. *J Clin Invest* 1962; 41:401-410.
12. Bosman AR, Honour AJ, Lee G de J, et al: A method for measuring instantaneous pulmonary capillary blood flow and right ventricular stroke volume in man. *Clin Sci* 1964; 26:247-260.
13. Becklake MR, Varvis CJ, Pengelly LD, et al: Measurement of pulmonary blood flow during exercise using nitrous oxide. *J Appl Physiol* 1962; 17:579-586.
14. Ayotte B, Seymour J, McIlroy MB: A new method for measurement of cardiac output with nitrous oxide. *J Appl Physiol* 1970; 28:863-866.
15. Carlin R, Chien S: Partition of xenon and iodoantipyrine among erythrocytes, plasma, and myocardium. *Circ Res* 1977; 40:497-504.
16. Carlin R, Chien S: Effect of hematocrit on the washout of xenon and iodoantipyrine from dog myocardium. *Circ Res* 1977; 40:505-509.
17. Kety SS, Schmidt CF: The nitrous oxide method for the quantitative determination of cerebral blood flow in man: Theory, procedure, and normal values. *J Clin Invest* 1948; 27:476-483.
18. Rowe GG: The nitrous-oxide method for determining coronary blood flow in man. *Am Heart J* 1959; 58:268-281.
19. Klocke FJ, Wittenberg SM: Methodological considerations in inert gas measurements of coronary blood flow, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 121-135.
20. Klocke FJ, Bunnell IL, Wittenberg SM, et al: Validation of inert gas measurements of coronary blood flow and contrasting findings in patients with and without coronary artery disease, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 321-332.
21. Klocke FJ: Coronary blood flow in man. *Prog Cardiovasc Dis* 1976; 19:117-166.
22. Tauchert M, Kochsiek K, Heiss HW, et al: Measurement of coronary blood flow in man by the Argon method, in Maseri A (ed): *Myocardial Blood Flow In Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 139-144.
23. Hoffman JIE, Buckberg GD: Transmural variations in myocardial perfusion, in Yu P, Goodwin JF (eds): *Progress in Cardiology*. Philadelphia, Lea & Febiger, 1976, vol 5, pp 37-89.
24. Ross RS, Ueda K, Lichtlen PR, et al: Measurement of myocardial blood flow in animals and man by selective injection of radioactive inert gas into the coronary arteries. *Circ Res* 1964; 15:28-41.
25. Maseri A: Myocardial flow by precordial residue detection following intracoronary slug injection of radioactive diffusible indicators, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 145-158.
26. Maseri A: Radioactive tracer techniques for evaluating coronary flow, in Yu P, Goodwin JF(eds): *Progress in Cardiology*. Philadelphia, Lea & Febiger, 1976, vol 5, pp 141-168.

27. McGregor M: Significance of myocardial flow measurement in the evaluation of the coronary patient, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 287–296.
28. Maseri A, Pesola A, L'Abbate A, et al: Contribution of recirculation and fat diffusion to myocardial washout curves obtained by external counting in man: Stochastic versus monoexponential analysis. *Circ Res* 1974; 35:826–834.
29. L'Abbate A, Maseri A, Ballestra AM, et al: Stochastic and exponential analysis of precordial washout curves for myocardial blood flow measurement: experimental evaluation. *Circ Res* 1981; 49:41–51.
30. Cannon PJ, Dell RB, Dwyer EM Jr: Measurement of regional myocardial perfusion in man with ^{133}Xe and a scintillation camera. *J Clin Invest* 1972; 51:964–977.
31. Cannon PJ, Weiss MB, Sciacca RR: Myocardial blood flow in coronary artery disease: Studies at rest and during stress with inert gas washout techniques. *Prog Cardiovasc Dis* 1977; 20:95–120.
32. Falseti HL, Carroll RJ, Marcus ML: Temporal heterogeneity of myocardial blood flow in anesthetized dogs. *Circulation* 1975; 52:848–853.
33. Sestier FJ, Mildenerger RR, Klassen GA: Role of autoregulation in spatial and temporal perfusion heterogeneity of canine myocardium. *Am J Physiol* 1978; 235:H64–H71.
34. King RB, Bassingthwaighte JB, Hales JRS, et al: Stability of heterogeneity of myocardial blood flow in normal awake baboons. *Circ Res* 1985; 57:285–295.
35. Sullivan JM, Taylor WJ, Elliot WC, et al: Regional myocardial blood flow. *J Clin Invest* 1967; 46:1402–1412.
36. Aukland K, Bower BF, Berliner RW: Measurement of local blood flow with hydrogen gas. *Circ Res* 1964; 14:164–187.
37. Aukland K, Kiil F, Kjekshus J, et al: Local myocardial flow measured by hydrogen polarography; distribution and effects of hypoxia. *Acta Physiol Scand* 1967; 70:99–111.
38. Selwyn AP, Jones T, Turner JH, et al: Continuous assessment of regional myocardial perfusion in dogs using krypton-81m. *Circ Res* 1978; 42:771–777.
39. Jones T: The steady state radioisotopic measurement of regional tissue blood flow, in Mathis RT (ed): *Blood Flow Measurement in Man*. Tunbridge Wells, Castle House Publications, 1982, pp 28–36.
40. Kety SS: Theory of blood-tissue exchange and its application to measurement of blood flow, in Bruner HD (ed): *Methods in Medical Research*. Chicago, Year Book Publishers, 1960a, vol 8, pp 223–236.
41. Utley J, Carlson EL, Hoffman JIE, et al: Total and regional myocardial blood flow measurements with 25 micron, 15 micron, 9 micron, and filtered 1-10 micron diameter microspheres and antipyrine in dogs and sheep. *Circ Res* 1974; 34:391–405.
42. Little SE, Bassingthwaighte JB: Plasma-soluble marker for intraorgan regional flows. *Am J Physiol* 1983; 245:H707–H712.
43. Sapirstein LA: Fractionation of the cardiac output of rats with isotopic potassium. *Circ Res* 1956; 4:689–692.
44. Sapirstein LA: Regional blood flow by fractional distribution of indicators. *Am J Physiol* 1958; 193:161–168.
45. Strauss HW, Pitt B: Myocardial perfusion imaging in the evaluation of patients

- with coronary heart disease, in Yu PM, Goodwin JF (eds): *Progress in Cardiology*. Philadelphia, Lea & Febiger, 1976, vol 5, pp 169–182.
46. L'Abbate A, Biagini A, Michelassi C, et al: Myocardial kinetics of thallium and potassium in man. *Circulation* 1979; 60:776–785.
 47. Schelbert HR, Henze E, Phelps ME: Emission tomography of the heart. *Sem Nucl Med* 1980; 10:355–373.
 48. Budinger TF, Yano Y, Huesman RH, et al: Positron emission tomography of the heart. *Physiologist* 1983; 26:31–34.
 49. Schelbert HR, Phelps ME, Huang S-C, et al: N-13 ammonia as an indicator of myocardial blood flow. *Circulation* 1981; 63:1259–1272.
 50. Schelbert HR, Phelps ME, Hoffman FJ, et al: Regional myocardial perfusion assessed with N-13 labelled ammonia and positron emission computerized axial tomography. *Am J Cardiol* 1979; 43:209–218.
 51. Rhodes CG, Lenzi GL, Frackowiak RSJ, et al: Measurement of CBF and $C^{15}O_2$ using the continuous inhalation of $C^{15}O_2$ and $^{15}O_2$ - experimental validation using CO_2 reactivity in the anaesthetised dog. *J Neurol Sci* 1981; 50:381–389.
 52. Allan RM, Selwyn AP: Myocardial blood flow measurement in man, in Mathie RT (ed): *Blood Flow Measurement in Man*. Tunbridge Wells, Castle House Publications, 1982, pp 83–98.
 53. Rutishauser W: Usefulness and limitations in roentgendensitometric coronary artery blood flow measurements, in Maseri A (ed): *Myocardial Blood Flow in Man: Methods and Significance in Coronary Disease*. Torino, Minerva Medica, 1972, pp 175–181.
 54. Bursch JH, Ritman EL, Wood EH, et al: Roentgen videodensitometry, in Bloomfield DA (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 313–333.
 55. Boyd DP, Gould RG, Quinn JR, et al: A proposed dynamic cardiac 3-D densitometer for easy detection and evaluation of heart disease. *IEEE Trans Nucl Sci* 1979; NS-26:2724–2727.
 56. Baer RW, Payne BD, Verrier ED, et al: Increased number of myocardial blood flow measurements with radionuclide labeled microspheres. *Am J Physiol* 1984; 246:H418–H434.
 57. Rudolph AM, Heymann MA: Circulation of the fetus in utero: Methods for studying distribution of blood flow, cardiac output, and organ blood flow. *Circ Res* 1967; 21:163–184.
 58. Makowski EL, Meschia G, Droegemueller W, et al: Measurement of umbilical arterial blood flow to the sheep placenta and fetus in utero. *Circ Res* 1968; 23:623–631.
 59. Archie JP Jr, Fixler DE, Ulliyot DJ, et al: Measurement of cardiac output with and organ trapping of radioactive microspheres. *J Appl Physiol* 1973; 35:148–154.
 60. Hales JRS: Radioactive microsphere measurement of cardiac output and regional tissue blood flow in sheep. *Pflügers Arch* 1973; 344:119–132.
 61. Buckberg GD, Luck JC, Payne BD, et al: Some sources of error in measuring regional blood flow with radioactive microspheres. *J Appl Physiol* 1971; 31:598–604.
 62. Dole WP, Jackson DL, Rosenblatt JL, et al: Relative error and variability in blood flow measurements with radio-labelled microspheres. *Am J Physiol* 1982; 243:H371–H378.

63. Domenech RJ, Hoffman JIE, Noble MIM, et al: Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs. *Circ Res* 1969; 25:581-596.
64. Yipintsoi T, Dobbs WA Jr, Scanlon PD, et al: Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dog hearts. *Circ Res* 1973; 33:573-587.
65. Fan FC, Schuessler GB, Chen RYZ, et al: Determinations of blood flow and shunting of 9- and 15- μ m spheres in regional beds. *Am J Physiol* 1979; 237:H25-H33.
66. Consigny PM, Verrier ED, Payne BD, et al: Acute and chronic microsphere loss from canine left ventricular myocardium. *Am J Physiol* 1982; 242:H392-H404.
67. Heymann MA, Payne BD, Hoffman JIE, et al: Blood flow measurements with radionuclide-labeled particles. *Prog Cardiovasc Dis* 1977; 20:55-79.
68. Hoffman JIE, Payne BD, Heymann MA, et al: The use of microspheres to measure blood flow, in Linden RJ (ed): *Techniques in Cardiovascular Physiology: Part I*. New York, Elsevier North-Holland, 1983, p 304, pp 1-36.
69. Maseri A, Mancini P, Contini C, et al: Method for the estimate of total coronary flow by 99 Tc tagged albumin microspheres. *J Nucl Biol Med* 1971; 15:58-60.
70. Wagner HN Jr, Sabiston DRJ, Iio M: Regional pulmonary blood flow in man by radioisotope scanning. *JAMA* 1964; 187:601-603.
71. Wagner HN Jr, Sabiston DC Jr, McAfee JG, et al: Diagnosis of massive pulmonary embolism in man by radioisotope scanning. *N Engl J Med* 1964; 271:377-384.
72. Tow DE, Simon AL: Comparison of lung scanning and pulmonary angiography in the detection and follow-up of pulmonary embolism: The Urokinase-pulmonary embolism trial experience. *Prog Cardiovasc Dis* 1975; 17:239-245.
73. Friedman WF, Braunwald E, Marrow AG: Alterations in regional pulmonary blood flow in patients with congenital heart disease studied by radioisotope scanning. *Circulation* 1968; 37:747-758.
74. Gates GF: *Radionuclide Scanning in Cyanotic Heart Disease*. Springfield, IL, Charles C Thomas, Publisher, 1974.
75. Reivich M, Jehle J, Sokoloff L, et al: Measurement of regional cerebral blood flow with antipyrine ^{14}C in awake cats. *J Appl Physiol* 1969; 27:296-300.
76. Bassingthwaighe JB, Strandell T, Donald DE: Estimation of coronary blood flow by washout of diffusible indicators. *Circ Res* 1968; 23:259-278.
77. Yipintsoi T, Bassingthwaighe JB: Circulatory transport of iodoantipyrine and water in the isolated dog heart. *Circ Res* 1970; 27:461-477.

Indicator Dilution Techniques

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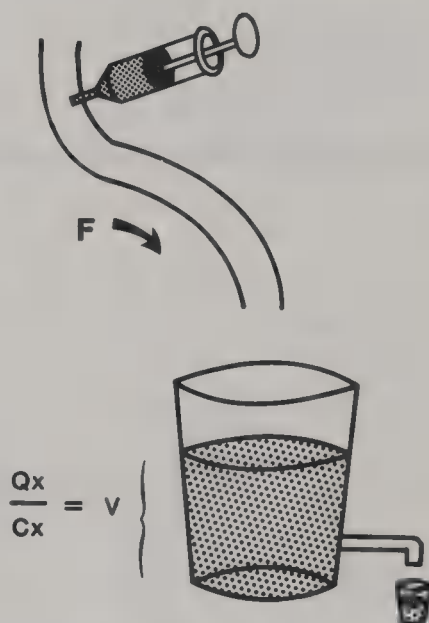
As described in detail with the discussion of the Fick principle (in the previous chapter), when a mass of an indicator is added at a constant rate to a stream of blood, a steady state is reached and the flow rate of blood can be determined from the constant inflow and outflow concentrations of the indicator and the rate it is added.¹⁻³ On the other hand when the indicator is injected as a bolus steady state concentrations are not attained, but the blood flow can be calculated from the amount of indicator added and the concentrations of indicator leaving the system. This is the principle of indicator or dye dilution and it provides an extremely useful technique for measuring blood flow to regions of the circulation as well as the entire cardiac output. Moreover, it has particular clinical application for measurement of cardiac output when: (1) oxygen consumption cannot be readily measured (e.g., when inspired oxygen concentration is high,⁴ the patient is mechanically ventilated,⁵ and expired gas cannot be collected), (2) there are cardiovascular shunts and no appropriate mixing site for sampling, and (3) bedside computation of flow is desired. We will review the general principles and sources of error and then consider the two most commonly used indicators for clinical application. Finally, for the interested reader we have included in an appendix some variations of the general approach which provide the basis for measuring other aspects of circulatory function.

Let us consider a fluid flowing through a tube at a constant rate. When a quantity of indicator is added as a bolus, the concentration of the indicator in the fluid is transiently changed, i.e., it increases abruptly and gradually decreases. If the indicator concentration is sampled far enough downstream from the site of the injection to permit complete mixing, the flow rate can be calculated from a mass balance of the indicator entering and leaving the system.

Before describing the method and assumptions for calculating flow rate *in vivo*, first consider a more simple system. As shown in Figure 4, the fluid to which the indicator is added passes through a hose and is collected in a bucket for a time sufficient to recover all the indicator. Since all of the indicator mixes with the volume collected in that period of time (t) there is a single mean concentration (C_x) of the indicator in the volume. From the amount of indicator added (Q_x) and the concentration, the volume (V) in the bucket can be calculated: $V = Q_x/C_x$. The flow rate can then be

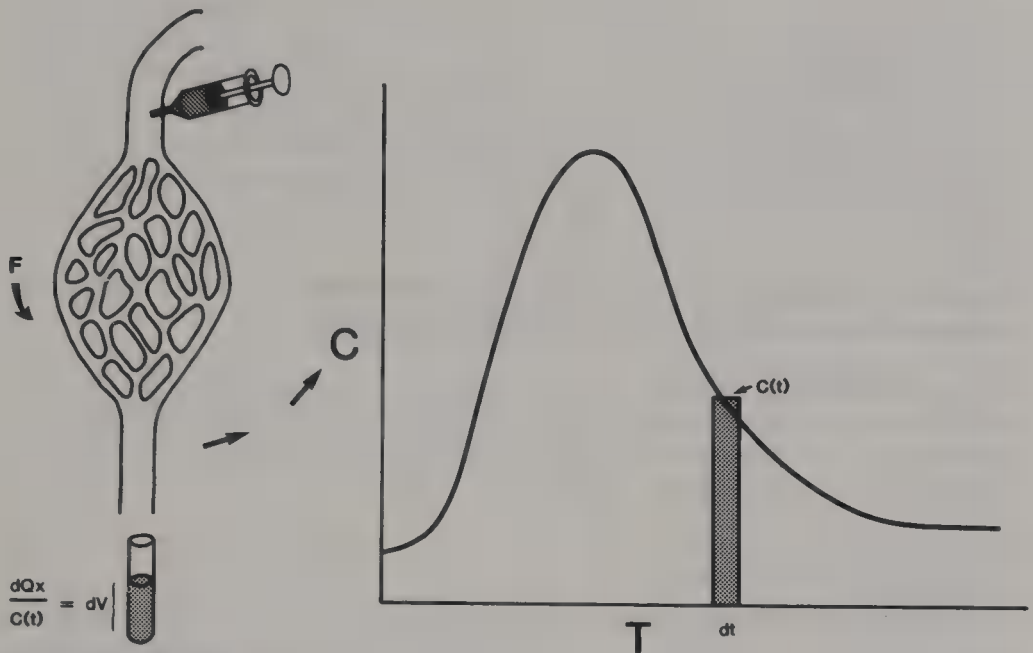
FIG 4.

Blood flow measurement with indicator dilution technique when all the dye is collected. As described in the text, this figure represents a bolus injection of an indicator into a stream and collection of all the indicator in a bucket. The concentration of dye in the bucket is identical to the concentration in a small aliquot collected from a spigot. The volume (V) in the bucket is equivalent to the amount of dye (Q_x) injected divided by the concentration (C_x). See text for description of flow (F) calculation.



determined from the volume and time of collection: $F = V/t$. When terms are rearranged, flow can be calculated without actually measuring volume directly: $F = (Q_x/C_x)/(t) = Q_x/C_x t$. Moreover all of the fluid need not be collected. A spigot could be connected to the bucket and a small fraction of fluid with indicator collected. The final mean concentration should be the same as in the bucket, and again the total flow rate could be calculated. This is the essence of the calculation of flow rate *in vivo* with the bolus injection except that all the fluid is generally not collected and a single mean concentration is not obtained, rather the changing concentration is measured as a function of time. Then the concentrations during a series of short time periods is multiplied by the time and all the products are summed (the integral of $C_x dt$).

Consider now that a bolus of indicator is injected into the inflow of a vascular system and the concentration of the indicator is measured continuously downstream as shown in Figure 5. During a very small time interval, a small volume of blood exits the system and is collected while the flow rate is constant; this volume is equivalent to the product of flow (F) and the time interval for collection (dt): $dV = F dt$. The volume of blood, dV , also contains a fraction (dQ_x) of the total (Q_x) indicator injected, which is equivalent to dV times the concentration of indicator ($C(t)$) during that time interval: $dQ_x/dV = C(t)$ or $dQ_x = dV C(t)$. Therefore, $dQ_x = F dt C(t)$ or $F C(t) dt$. If we sum all of the fractions of indicator exiting over time it should total Q_x , the amount of the indicator injected. Similarly if we sum all the products of concentration at any given time and the volume collected in that time this yields the integral, $\int_0^\infty F dt C(t)$ or $F \int_0^\infty C(t) dt$.

**FIG 5.**

Indicator dilution technique with the concentration measured continuously. As described in the text, flow rate (F) can be measured through a vascular bed after the injection of an indicator upstream and continuous measurement of concentration (C) as a function of time (T) downstream. The concentration at any point in time ($C(t)$) shown by the shaded area is equivalent to the concentration that will be obtained if an aliquot of fluid and dye were collected for a very short time (dt); the aliquot is shown by the small test tube with a volume (dV), which is equal to the amount of dye collected (dQ_x) divided by the concentration ($C(t)$). See text for description of calculation of blood flow.

This integral is equivalent to the amount of indicator injected ($Q_x = F \int_0^\infty C(t)dt$). Then, by rearranging terms, flow can be calculated as

$$F = \frac{Q_x}{\int_0^\infty C(t)dt} \quad (1)$$

This now provides the working equation to compute flow through a system when an indicator is added by bolus. Again as with the example of collection in a bucket, it is not necessary to sample the concentration in the main stream of blood. This will be discussed in detail later. An assumption here is that the concentration of the indicator initially entering the system was zero and all the indicator was added by the bolus. If some indicator was present in the stream to which the bolus of indicator was added, then the concentration of indicator entering the system must be

accounted for in the equation. This is the case with recirculation of the indicator, repeated measurements, or with thermodilution measurement (the blood enters with some heat or caloric content). When this inflow concentration of indicator is a constant term, the calculation is no more complex than equation 1. When this inflow concentration varies with time, computation of flow may become complicated. Therefore, it is generally desirable to choose indicators that are rapidly cleared from the circulation.

General Assumptions and Sources of Error

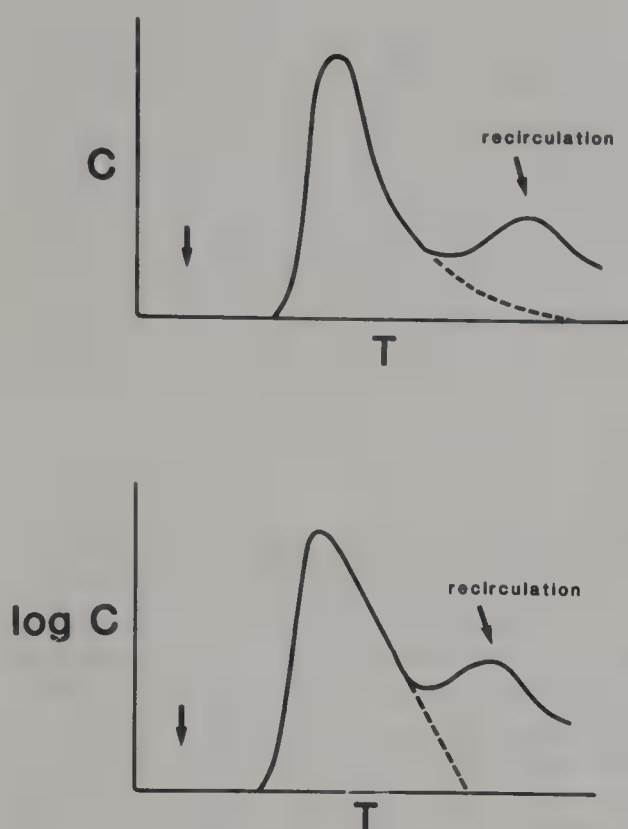
The use of indicator dilution techniques requires many assumptions. Here we will consider the general assumptions related to the technique and their implications, and later we will discuss the problems unique to specific indicators.

Conservation of Mass

For inferences to be drawn with regard to flow through a system it is essential that all the indicator be accounted for. For *in vivo* systems, it may be impractical or difficult to determine the point at which the concentration of the indicator has returned to zero (implying that all the indicator has been collected) following an injection. Time for sampling or blood withdrawal may be a constraint, or with recirculation, the dye may reappear before there is complete washout from the initial injection. The "true" indicator concentration can be accounted for by a variety of techniques.^{6,7} This is most easily obtained from the downslope or "washout" of the indicator dilution curve that generally follows an exponential decay. The log of the measured concentrations is plotted as a function of time. Additional points are estimated by extrapolation as shown in Figure 6. Many machines used for measurement of blood flow by indicator dilution are equipped with computers for performing this extrapolation but it is important to be aware of the assumptions used. For example, if recirculation is not recognized the area under the initial curve would be falsely overestimated resulting in an erroneously low blood flow calculation.

Mixing of Indicator

The assumption is made that all the indicator is well mixed, that the concentration measured downstream is representative of mass in the volume sampled, and that the movement of indicator (convection) is similar to the movement of the fluid. It may take time for the indicator to become well mixed. For example, indocyanine green dye (ICG) needs to be bound to protein and there is a requisite change in the spectral absorption that takes a few seconds.⁸ With all indicators, it may be necessary for the mixture to pass through a cardiac chamber or the vascular bed of an organ to avoid streaming at the site of sampling. Streaming occurs even in long blood

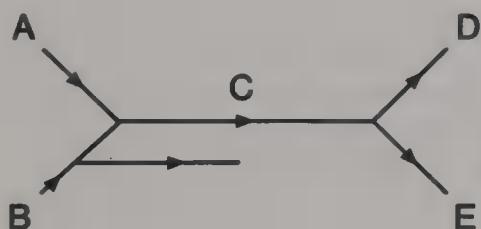
**FIG 6.**

The relationship between the concentration of indicator (C) and time (T) and the log concentration of indicator ($\log C$) and time (upper and lower panels, respectively). This figure shows the concentration of indicator as it is obtained in a system with recirculation after a bolus injection (arrow). Without recirculation, the indicator concentration would decrease to zero as shown by the dotted line. The theoretical "true" indicator concentration that would be obtained with no recirculation can be derived from a semi log plot as shown on the bottom panel. The dotted line now shows extrapolation to provide the expected concentration of dye.

vessels (such as the inferior vena cava or aorta) unless there is an interposed mixing chamber.⁹ For measurement of cardiac output, this problem may be solved by an injection into the right atrium and sampling in the pulmonary artery or at a more distal site, or injection in the pulmonary artery and sampling on the left side of the heart.

Number of Inlets and Outlets

The sites of sampling and injection are particularly important to consider. Figure 7 helps illustrate some of the concerns raised here. Blood flow can be assessed in a system with multiple inlets, multiple outlets, or both. However, it is essential to recognize what flow will actually be measured when there is not a single inlet and outlet. If an injection is made at inlet A and sampling is at C, D, or E, the flow measured will be that through C. On

**FIG 7.**

A multiple inlet and outlet vascular system. See text for description of appropriate sites of injection and withdrawal for measurement of blood flow through channel C.

the other hand, if the injection is made at B, the flow that is measured from sampling at C, D, or E will be erroneously high because some of the injectate was "lost" before reaching C. These are the types of considerations that occur in the presence of shunts. For example, if there is a right-to-left atrial shunt and dye is injected at the right atrium and sampled in the pulmonary artery, the calculation will overestimate pulmonary blood flow. However, if there is a left-to-right atrial shunt and dye is injected at the right atrium and sampled in the pulmonary artery, the calculation will measure pulmonary blood flow. Needless to say, the presence of shunts often complicates the blood flow measurement, and it is worth the time to diagram flow patterns to determine the appropriate site for injection or sampling.

Disturbance of the System

Finally, it is important that the injection of the indicator not disturb the system. The system should be in steady state with regard to flow. This means that even if there is pulsatile blood flow, the heart rate or period of pulsatility should be relatively small compared to the time for sampling. Then the resultant flow will be the average for the time period sampled. The injection volume should be very small relative to the amount in the system so that flow rate is not altered by this injection. This is usually satisfied by having relatively concentrated indicators, or for example, an injectate that is much colder than blood. However, when dealing with organ blood flow or blood flow in small subjects this is an important consideration.

Cardiac Output Measurement

Indocyanine Green

In usual practice, the measurement of cardiac output using ICG as the indicator requires two indwelling catheters.^{10, 11} A catheter for injection is usually placed in the central venous circulation, right ventricle, or pulmonary artery and a catheter for withdrawal is placed in a systemic artery. Variations of site of injection and withdrawal can be used for detection and quantitation of shunts. In practice, it is easiest to fill the dead space of catheter with the dye and then inject by flushing with a small amount of saline or similar fluid. Thus, little fluid need be injected but all the dye clears the tubing. Blood is simultaneously withdrawn at a constant rate from the arterial catheter through a cuvette connected to a densitometer that measures optical density. The time for withdrawal varies depending on how far apart the sites of injection and withdrawal are but this can

easily be determined by some test runs. For small patients it is desirable to minimize the time for withdrawal because of the loss of blood during the measurement; it is also often necessary to adjust the amount of dye injected to keep the curve on scale. Finally, it is necessary to calibrate the system. This is done by putting known concentrations of dye into a measured volume of blood and determine the optical density for that concentration. Some practical suggestions to simplify these measurements are suggested by Rudolph.⁷ As mentioned earlier, computation of cardiac output can be done by plotting the outflow curve of concentration-time and determining the area beneath this or by interfacing this system with a computer.

Many nontoxic dyes have been tried as possible indicators to be diluted in blood. ICG has the particular advantage of binding to albumin so that it is excreted rapidly in the liver (with a half-life of about 5 minutes) and has a maximum spectral absorption at 805 nm so that there is no interference from oxyhemoglobin or deoxyhemoglobin. Therefore, ICG can be injected as a bolus and its concentration measured by the use of a densitometer through which blood is drawn at a constant rate. Because the dye is cleared rapidly, repeated measurements can be made with minimal interference from a background concentration. The advantages of this method are that it is safe, it is easy to quantify the dye in blood, and it can be used in conjunction with other markers to provide additional information. For example when ICG is used with a thermal indicator, extravascular water can be calculated as will be discussed in the appendix. Moreover, there is now equipment that can be used at the bedside that permits rapid calculation of cardiac output from the dye curve. In the past it would be necessary to calculate cardiac output by plotting outflow concentration in relationship to time and integrating the area under the curve (denominator of equation 1) using a planimeter or reconstructing the semilog plot as mentioned earlier. If one is to use an automated device, however, it is important to know what assumptions are made in the program for calculation, at what point extrapolation is started, and what curves will be rejected. Recently, intravascular sensors have been developed that permit measurement of spectral absorption without the need for blood withdrawal. This combined with a computed flow could greatly simplify cardiac output and possibly some regional blood flow measurements in patients but these systems require further validation.

An additional application of ICG as an indicator is its use for detecting, localizing, and quantifying cardiovascular shunts.^{7, 12} If there is a shunt between the site of dye injection and sampling, then the outflow curve contour provides information about the direction and magnitude of the shunt. When there is a shunt, the outflow curve becomes bimodal. A right-to-left shunt is characterized by an early peak (the shunt) that is superimposed on the upstroke of the major curve (the predominant flow through the circulation). With a left-to-right shunt, the extra peak appears on the downstroke of the major curve. In fact, a left-to-right shunt is analogous to the

shape of the curve with any recirculation, the differences are due to the magnitude of the additional peak and the rapidity with which it appears.

In general, the larger the area under the extra peak or the higher it is, the greater is the proportion of the shunt. A number of approaches have been used to quantify shunt fraction; some are relatively simple and rely on approximations of curve areas from the height of the peaks and others require more sophisticated curve-fitting techniques. However, because the curves from the shunt and the normal circulation are partially superimposed, it may be difficult to quantify the respective areas to determine the proportion of shunting, and the data obtained are often inconsistent. Although the magnitude of the shunt may not easily be determined, the site of the shunt can be localized by systematically changing the sites of injection and withdrawal, as described in detail elsewhere.⁸

There are some problems that need to be addressed. As mentioned before, it takes time for the spectrum to shift to 805 nm. Therefore, the site of sampling should be sufficient distance from the site of injection. The most practical approach is to inject into the right side of the heart and sample from the systemic artery. If there is reduced clearance of the ICG as with reduced liver blood flow, repeated measurements may be erroneous since the absorption of ICG at high concentrations may not be linear.¹¹ When there is a very low cardiac output the recirculation of coronary blood flow (which will be a proportionally larger fraction of cardiac output) will add error to the measurement.¹³

Most commonly, the method requires that blood be withdrawn from the subject. If sterile equipment is used, the blood may be reinfused; otherwise, blood loss must be a concern particularly for the small patient. Calibration also requires some withdrawal of blood since the dye needs to be diluted in blood to determine the relationship between concentration and absorption. Despite the technical and practical considerations, the use of ICG is a time-honored, reproducible, and well-established technique for measurement of blood flow and it has become more practical at the bedside with recent technical advances. Because of the potential for measurement of other functions of the vascular system, this approach will likely remain an important method for measurement of blood flow.

Thermal Dilution

A clever adaptation of the indicator dilution technique has been the use of calories as the indicator.^{10, 14-16} If a quantity of fluid is injected into a vascular system and that fluid has a temperature that differs from the blood then it will change the heat content of that blood by a predictable amount. The blood will also change its temperature in response to the bolus and the temperature change of the blood can be recorded with a thermistor. The quantity of heat injected is the numerator and the change in the quantity of heat in blood is in the denominator of the flow equation. Since it is

heat (or cold) that is being diluted, the specific gravity (S) and heat capacities (C) of both blood (B) and the injectate (i) must be known. Therefore,

$$F = \frac{(T_B - T_i) V_i \cdot C_i \cdot S_i}{C_B S_B \int_0^{\infty} \Delta T_B (t) dt}$$

where T_i and T_b are temperatures of injectate and blood and V_i is the volume of injectate. In general, if blood flow is diminished, a given bolus of cold will cause a large temperature change, and conversely, with high blood flow, a temperature change will be rather small. By using saline or glucose as the injectate at room temperature or 0 C (these are the two most practical temperatures for clinical purposes), the volume of the injectate can usually be limited to 1 to 5 ml for children. Under most circumstances, this provides a sufficient temperature change to be recorded and discern from the normal variability in blood temperature. In practice, a catheter (usually balloon flow-directed type such as a Swan-Ganz catheter) is used that has a thermistor at its tip and a hole 5 to 15 cm proximal to this. When placed in the right side of the circulation, the tip may be located in the pulmonary artery and the thermistor can record baseline blood temperature prior to injection. The injectate temperature is recorded prior to injection by a separate thermistor. Following a bolus injection the change in the blood temperature downstream can be recorded continuously and flow calculated on line or from a plot. Variations on this approach can be used. For example a thermistor may be placed on the descending aorta with an injection into the left atrium or left ventricle.

There are many clear advantages to this system. First, it is safe and the substances used for injection are obviously nontoxic. However, for the small patient, the volume of injectate can eventually be a problem if multiple measurements are made. For the infant, 1 ml of an iced injectate may be sufficient to produce an adequate indicator curve, but the smaller the injectate the more room for error because of the lost volume in the catheter. Another major advantage is that the indicator is rapidly eliminated. As the cold passes through small vascular beds, the temperature is distributed to tissues and body water. Therefore, in the absence of cardiac shunts, there is no appreciable recirculation. However, the lung tissue is also a sink for heat so that cardiac output cannot be measured by injecting in the pulmonary artery and sampling in the aorta.

Despite its many advantages, there are also theoretic and practical problems with the thermal method. The exact amount of calories injected is difficult to measure. As the bolus is injected and passes through the catheter cold is lost to the catheter and to the skin in a somewhat unpredictable fashion. Most devices or systems used for computing thermal dilution cardiac output have a fudge factor because of this lost cold. Next, there is baseline variability in blood temperature. Different vascular beds have different temperatures and there are temperature fluctuations under normal

conditions. Some of this may be eliminated by averaging, but under pathologic states these variations may be accentuated. It may also be difficult to determine when there has been a return to baseline. Because of the uncertainty of the return to baseline, integration of the curve may be difficult if the decay is very slow or the injection is not made rapidly. For repeated measurements, most automated systems have been programmed to have a time period of temperature stability that must be present before a second injection can be made.

In general, thermal dilution has proven to be a very useful and practical bedside technique. It eliminates the need for withdrawal of blood, which is a particular advantage in children. A single catheter can be used for injection and sampling and this can be part of a flow directed catheter so that multiple functions can be provided. Furthermore the injectate is non-toxic and the equipment is easy to use. Thus, both ICG and thermal dilution provide relatively straightforward, practical methods for obtaining measurement of cardiac output for clinical use with few hazards. In the appendix, some other applications of indicator dilution measurements are described.

Appendix: Other Applications for Indicator Dilution

Measurement of Mean Transit Time and Volume of Distribution

When an indicator is injected into a vascular system and sampled downstream, the outflow curve contains information about the volume of fluid in that system between the points of injection and sampling and the average time (mean transit time) it takes for the indicator particle to traverse the distance between those points.^{1, 17} To appreciate the derivation of this information, consider an injection of a dye that is mixed with the fluid upstream from the point of sampling and travels with the fluid through the system. The particles of dye take varying amounts of time to reach a site of sampling. This is particularly true if they must pass through the vascular bed of an organ where there may be different lengths of vessels to traverse or different flow rates within the vessels; some particles have short and others have long transit times. To calculate mean transit time for these particles or the fluid to traverse the vascular bed, certain general assumptions are necessary: (1) the distribution of traversal times for particles entering the system is constant with respect to time, (2) total flow rate through the system is constant, (3) volume in the system is constant, (4) all fluid that moves into the system eventually moves out, (5) the indicator does not recirculate during the period of time in which the measurement is made, and (6) the particles travel in a fashion representative of the total fluid flow. After the dye is injected, there is a delay before the first particle arrives at the site of sampling and this is called the appearance time. Fol-

lowing this the concentration of dye emerging from the system is a function of the amount of fluid labeled with dye that is draining relative to the total amount of fluid exiting the system. At the earliest points in time, fluid travelling through the shortest distances or at the most rapid rate will arrive at the point of sampling first. Much of the fluid initially exiting represents fluid that was already in the organ at the time the dye was injected and therefore it contains no dye. With time the concentration at the point of sampling increases and most of the fluid exiting has been labeled with dye.

The transit time for any given particle is the sum of the appearance time plus the time after the start of the curve before that particle exits. The average time it takes for the particles to exit the vascular system is a function of the time each particle takes to pass through the system and the total number of particles. Such an average could be obtained by summing the products of transit time and amount of dye exiting with that transit time and dividing this sum by the total amount of dye collected. In practice, concentration is measured rather than the amount of dye, and mean transit time is calculated as

$$\bar{t} = \text{appearance time} + \int_0^{\infty} t C(t) dt / \int_0^{\infty} C(t) dt \quad (2)$$

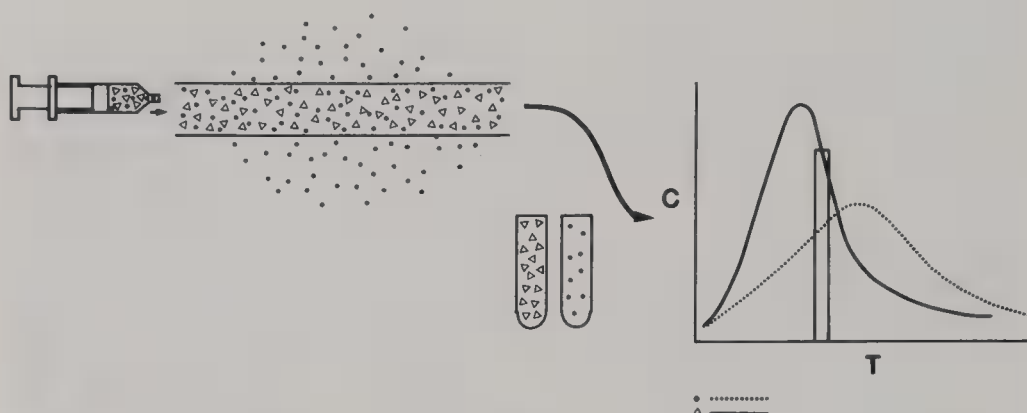
If we now consider that fluid traverses a vascular system at a given flow rate (F) and the average time (or mean transit time, \bar{t}) that an aliquot of fluid or a particle spends in the vascular system is known, then the volume (V) in that vascular system at any point in time is the product of these two values:

$$V = F \times \bar{t}$$

This volume of distribution represents the amount of fluid into which the indicator would disperse if the vascular flow were abruptly stopped and the entire system were mixed. Clearly, this could provide valuable information if one were interested in the blood volume of an organ and there were the ability to inject and sample at the inflow and outflow of that organ. This is particularly useful in an organ with inflow and outflow source that is relatively accessible, such as the lung.

Measurement of Extravascular Lung Water

If an indicator is injected into the vascular system and the indicator not only distributes within the blood volume but diffuses out of the blood vessels, the outflow curve will reflect this distribution into a larger volume (Fig 8).¹⁸⁻²⁰ If some of the indicator is lost from the vascular space, the concentration in blood at any given point in time will be lower and the apparent blood flow will be greater. Although this blood flow measurement is erroneous, the particles that remain in the vascular space still have a distribution of transit times that is uninfluenced by the loss of indicator. Therefore, the mean transit time will be correct and a new volume of distribution

**FIG 8.**

Concentration (C) shown as a function of (T) after injection of a mixture of two indicators given in equal quantities into a vascular system. The intravascular marker (Δ) stays entirely within the vascular space and yields a high concentration during the early part of the outflow curve as shown by the solid line or the high concentration in an aliquot that is collected for a short period of time. The diffusible marker (\cdot) distributes in both the intravascular and extravascular space; therefore, its concentration within the intravascular space is lower during the early part of the outflow curve.

can be calculated that will include all the space into which the indicator moved. As before, the concept still holds that the average concentration of the indicator in the vascular space is that which would be obtained if the indicator were injected and all flow were stopped. Since the indicator moves freely from the vascular space into the extravascular space, the volume of distribution now is the sum of those two. Also if an indicator quickly distributes in extra as well as intravascular water, the volume of extravascular water (V_{dEVLW}) can be measured by subtracting the volume of distribution of an intravascular marker (V_{di}) from that of the marker that diffuses (V_{dH_2O}) into both spaces:

$$V_{dH_2O} - V_{di} = V_{dEVLW}$$

or

$$V_{dEVLW} = \dot{Q} (\bar{t}_{H_2O}) - \dot{Q} (\bar{t}_i) = \dot{Q} (\bar{t}_{H_2O} - \bar{t}_i)$$

where \dot{Q} is the plasma water flow and \bar{t}_{H_2O} and \bar{t}_i are the mean transit times for water and the intravascular markers, respectively. This is the principle behind the use of simultaneous injection of two markers into the pulmonary circulation. Usually ICG is combined with a bolus of (labeled) water and the two are measured at the outflow of the pulmonary circulation, or in the aorta. The water may be labelled with deuterium, tritium, or injected as a thermal indicator. In theory, the volume of extravascular water measured may only represent that which resides near perfused portions

of the lung and is accessible to the extravascular marker. Therefore, this technique frequently underestimates water measured gravimetrically. In practice, there are other sources of error common to all indicator methods, but it provides an interesting approach to an important physiologic variable.

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References

1. Lassen NA, Henriksen O, Sejrsen P: Indicator methods for measurement of organ and tissue blood flow, in Shepherd JT, Abboud FM (eds.): *Handbook of Physiology*. Section 2: *The Cardiovascular System*, vol III, part 1. Bethesda, American Physiological Society, 1983, pp 21–63.
2. Antman S: Foundations of indicator-dilution theory, in Bloomfield DA (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 21–40.
3. Lassen NA, Perl W: *Tracer Kinetic Methods in Medical Physiology*. New York, Raven Press, 1979.
4. Welch HG, Pedersen PK: Measurement of metabolic rate in hyperoxia. *J Appl Physiol* 1981; 51:725–731.
5. Abdul-Rasool IH, Chamberlain JH, Swan PC, et al: Measurement of respiratory gas exchange during artificial respiration. *J Appl Physiol* 1981; 51:1451–1456.
6. Bloomfield DA: The calculation of curve areas, in Bloomfield DA (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 71–86.
7. Rudolph AM: *Congenital Diseases of the Heart*. Chicago, Year Book Medical Publishers, Inc, 1974, pp 140–149.
8. Saunders KB, Hoffman JIE, Noble MIM, et al: A source of error in measuring flow with indocyanine green. *J Appl Physiol* 1970; 28:190–198.
9. Miller HC, Brown DJ, Miller GAH: Comparisons of formulae used to estimate oxygen saturation of mixed venous blood from caval samples. *Br Heart J* 1974; 36:446–451.
10. Bloomfield DA: A method for performing an indicator-dilution curve to measure cardiac output, in Bloomfield DH (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 41–53.
11. Tripp MR, Swayze CR, Fox IJ: Indocyanine green, in Bloomfield DA (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 365–391.

12. Krovetz LJ: Detection and quantification of intracardiac and great vessel shunts, in Bloomfield DA (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 119–143.
13. Oril A, Sekelj P, McGregor M: Limitations of indicator-dilution methods in experimental shock. *J Appl Physiol* 1967; 23:605–608.
14. Ganz W, Swan JHC: Measurement of blood flow by the thermodilution technique, in Bloomfield DA (ed): *Dye Curves: The Theory and Practice of Indicator Dilution*. Baltimore, University Park Press, 1974, pp 245–266.
15. Vliers ACAP, Visser KR, Zijlstra WG: Analysis of indicator distribution in the determination of cardiac output by thermal dilution. *Cardiovasc Res* 1973; 7:125–132.
16. Moodie DS: Measurement of cardiac output by thermodilution in pediatric patients. *Pediatr Clin N Am* 1980; 27:513–523.
17. Zierler KL: Circulation times and the theory of indicator-dilution methods for determining blood flow and volume, in Hamilton WF, Dow P (eds): *Handbook of Physiology*. Section 2; *Circulation*, vol 1. Bethesda, American Physiological Society, 1962, pp 585–615.
18. Snapper JR, Harris TR, Brigham KL: Effect of changing lung mass on lung water and permeability-surface area in sheep. *J Appl Physiol* 1982; 52:1591–1597.
19. Harris TR, Brigham KL: The exchange of small molecules as a measure of normal and abnormal lung microvascular function. *NY Acad Sci* 1982; 384:417–433.
20. Lewis FR, Elings VB, Hill SL, et al: The measurement of extravascular lung water by thermal-green dye indicator dilution, in Malik AB, Staub NC (eds): *Mechanisms of Lung Microvascular Injury*. New York, Annals of the New York Academy of Science, vol 384, 1982, pp 394–410.

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Principles

Flow Velocity Determination

The Doppler principle is named for its discoverer, Christian Doppler, an Austrian physicist who noted in the early 19th century that sound reflected from a moving object underwent a frequency shift in the process. This phenomenon was applied first to the quantification of blood flow about 25 years ago, approximately 100 years after its description. The principles of ultrasonic measurement of blood flow velocity and the calculation of flow (Doppler flowmetry) will be summarized prior to discussion of specific applications to the quantification of central and regional blood flow.¹⁻⁴

Doppler devices employ sound in the frequency range of 1 to 20 million cycles per second (MHz). The range for audible sound is 20 to 20,000 Hz; therefore, Doppler units utilize what is referred to as *ultrasound*. Sound travels in wave forms, with a wave length = λ and a frequency = f . The speed of sound, 1,540 m/sec in blood, is represented as c , and $c = f \lambda$. As sound travels through a medium, its intensity is reduced or attenuated, due to reflection, beam divergence, and absorption (conversion to heat). A portion of the beam is *back-scattered* ("reflected") from objects in the beam pathway. In blood, red blood cells are the primary back-scattering structures, and between hematocrits of 10% to 70%, the intensity of back-scattered energy is a function primarily of the fourth power of the emission frequency, f_o .⁵

Quantification of red blood cell velocity by ultrasound depends on the Doppler effect. That physical principle relates the change in frequency (Δf) of sound back-scattered from an object moving at a velocity, V , by the equation

$$\Delta f = (2f_o V \cos \theta) / c$$

where θ is the intercept angle between the ultrasound beam and the axis of the flow stream—the angle of insonance. By rearranging the equation to

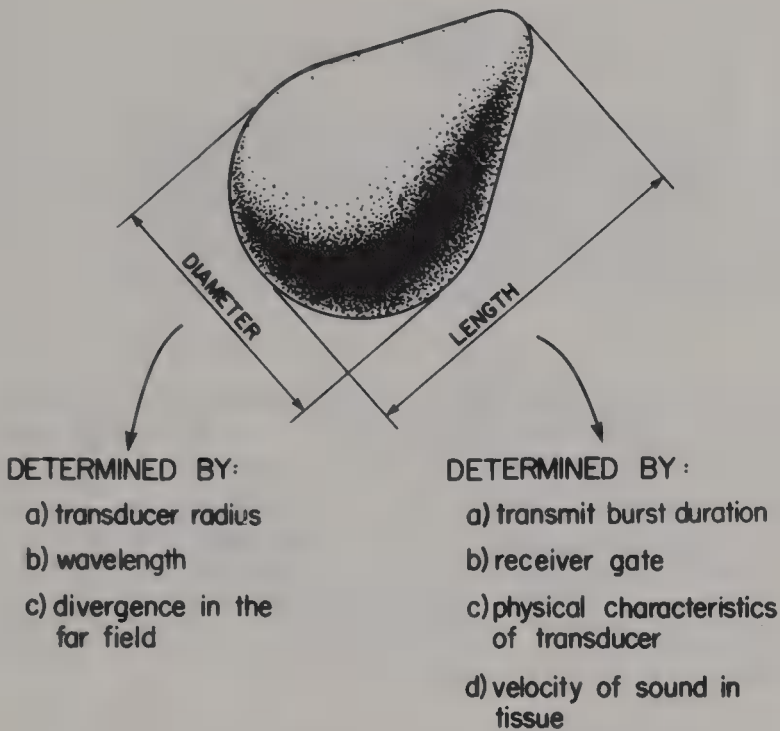
$$V = (c \Delta f) / (2f_o \cos \theta)$$

one can see that measured velocity depends on the emission frequency (f_o) and the velocity of sound in tissue (c) and varies directly with the

frequency shift (Δf) and inversely with the cosine of θ . In practice, two types of Doppler "systems" can be employed, continuous wave and pulsed. I will describe both and then focus on pulsed Doppler application in the remainder of the discussion.

Sound wave transducers employ piezoelectric crystals to both generate and receive ultrasound signals. With continuous wave systems, separate transducers continuously emit and receive ultrasound signals. As with all transducers, beam width varies directly with transducer diameter, emission frequency, and depth of penetration; penetration capabilities vary inversely with emission frequency. In current practice, received frequency shifts of 0 to 10 kHz reflect interface velocities of 0 to 100 cm/sec. With continuous emission and sampling, a spectrum of frequency shifts is obtained, as no gating procedure is used to discriminate any part of the field transected by the ultrasound beam. As such, the continuous wave system samples indiscriminantly, without specificity of range or depth. The wide range capability of continuous wave devices make them useful for detecting very high velocity streams, a property that has proven useful for estimation of vascular pressure gradients in clinical practice.⁶⁻⁹ The lack of range specificity imposes constraints related to sampling of all flow streams in the beam path.

By use of range gating, the Doppler transducer can be converted to a pulsed device, used both for emission and reception of ultrasound signals. The transducer emits bursts at a specified frequency—the pulse repetition frequency, or PRF—and is free to receive signals for the period between bursts, which is the sampling interval. If a 5-MHz transducer with a PRF of 10 kHz emits pulses of 1 μ sec in duration, then each pulse contains five wave cycles (computed as follows: $1 \times 10^{-6} \text{ sec} \times 5 \times 10^6 \text{ cycles/sec}$) spaced 100 μ sec apart ($1/\text{PRF} = 1 \text{ ten-thousandth of a second}$). The sampling time in this example is 99 μ sec, 99 times the transmission time; because of this gated operation, pulsed Doppler units deliver less ultrasound energy to tissue than continuous wave units of comparable emission frequency operated for an equal time.¹⁰ Since the velocity of ultrasound in biologic tissue is known (1.54 m/sec or 1.54 mm/ μ sec), the transmission time to and from a back-scattering target can be used to calibrate sampling depth or direct the instrument's sample volume (volume of interrogation) to a desired point. This axial or depth resolution is achieved by varying the sampling interval, and the dimensions of the sample volume itself are determined by physical factors, including transducer size, emission wave length, transmission duration, and the fields sampled (Fig 9). In the example cited, with the sampling interval = 99 microseconds, assuming equal transmission time to and from the back-scattering target, depth of sampling = $c \times (\text{sampling interval}/2) = 1.54 \text{ mm}/\mu\text{sec} \times 49.5 \mu\text{sec} = 76 \text{ mm or } 7.6 \text{ cm}$. Because this relationship of travel time to distance is nearly constant in soft tissue, one can use the 13 μ sec/cm calibration factor to determine sampling depth from sampling interval. This is the gating feature all pulsed Doppler units employ to achieve depth specificity or reso-

**FIG 9.**

Variables that affect the size and shape of the volume sampled by a pulsed Doppler system. (From Berman W, Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983. Used by permission.)

lution. This feature permits selective sampling, but also imposes constraints on the measurement of flow velocity of red blood cells.

For accurate processing of received ultrasound signals, the Shannon sampling theory requires the sampling frequency to be at least twice the highest frequency shift measured.¹¹ Expressed mathematically, $\Delta f_{\max} = \text{PRF}/2$. The PRF also limits the range of sampling, as back-scattered signals from the region of interrogation must have time to return to the transducer before a new pulse is emitted. The maximum range, R_{\max} , is related directly to c and inversely to PRF, as

$$R_{\max} = (c \times 10^{-1}) / (2 \times \text{PRF})$$

where PRF is measured in kHz, c in m/sec and R_{\max} in cm. If one substitutes these relationships of Δf_{\max} and R_{\max} in the Doppler equation, the limits of velocity and range determination can be calculated:

$$\begin{aligned} \Delta f &= (2f_o V \cos \theta) / c \\ \Delta f_{\max} &= \text{PRF} / 2 \end{aligned}$$

therefore,

$$\text{PRF} / 2 = (2f_o V_{\max} \cos \theta) / c$$

and,

$$V_{\max} = (c \times \text{PRF} \times 10^{-3}) / 4f_o \cos\theta$$

where V_{\max} and c are measured in meters/second, f_o is in MHz, and PRF is in kHz. From this equation, one notes the maximum unambiguously measurable velocity is directly proportional to PRF and inversely proportional to emission frequency. Since $R_{\max} = (c \times 10^{-1}) / (2 \times \text{PRF})$, one may substitute above for PRF as $(c \times 10^{-1}) / (2 \times R_{\max})$ to derive the following relationship:

$$V_{\max} = (c^2 \times 10^{-4}) / 8f_o \cos\theta R_{\max}$$

Here, one can note that the maximum measurable velocity varies inversely with the depth at which it is sampled. From these equations, one can calculate a range of velocities and sampling depths that a Doppler unit with known specifications can measure accurately and *unambiguously*. Beyond those limits, ambiguities of range or frequency shift (aliasing) occur. For a pulsed Doppler device with a 5-MHz transducer and a PRF of 10 kHz, Figure 10 can be drawn. The effects of variation in f_o on the relationship are shown in Figure 11: the lower the emission frequency, the greater the penetration possible for measuring a given V_{\max} .

Volumetric Flow Calculation

Flow calculation from flow *velocity* measurement rests on simple algebraic and geometric relationships. The calculation presumes that flow velocity in the sampled vessel is uniform (flat flow velocity profile), that the vessel is round (vessel area = πr^2 or $\pi d^2/4$), that the vessel diameter does not change during the flow cycle, and that an average flow velocity measurement (the temporal mean velocity, \bar{V}) can be made. With these assumptions and presumptions, volumetric flow (Q) = \bar{V} cm/sec \times $\pi d^2/4$ cm² = ml/sec. A review of the potential systematic and experimental errors in this calculation is essential prior to discussion of application of this measurement method.

Errors in Flow Calculation

Sampling Resolution

Many units for pulsed Doppler processing are part of imaging systems (duplex scanners) and use a single transducer for imaging and flow velocity determination. In pediatrics, resolution of small structures often requires a relatively high emission frequency (5 to 10 MHz) that limits penetration and V_{\max} determination (see Fig 11). As will be discussed later, instrument specifications must be matched to application to avoid ambiguities and to maximize measurement capabilities.

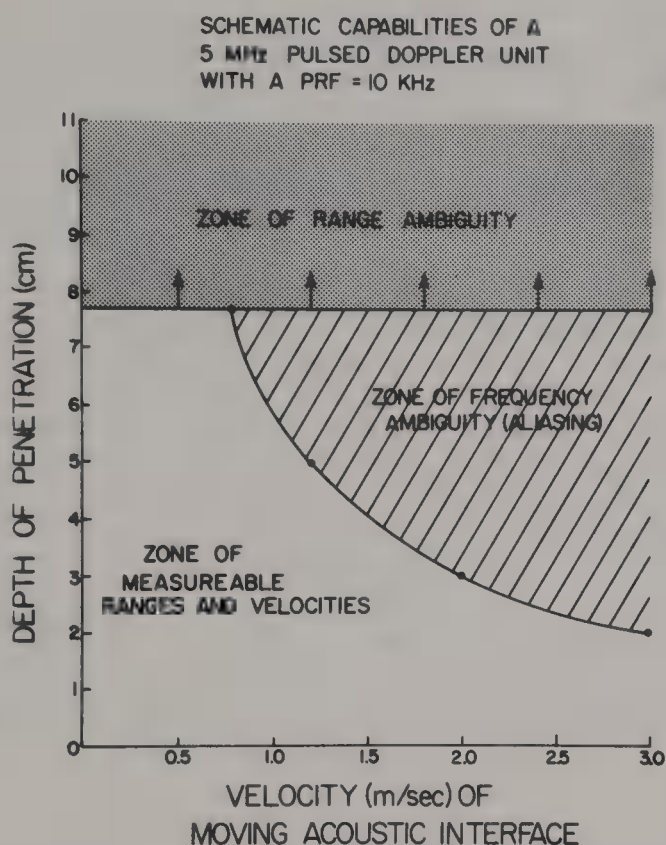


FIG 10.

Variables that determine the zone of unambiguous sampling for a pulsed Doppler system. (From Berman W, Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983. Used by permission.)

Signal Processing

A detailed review of signal processing will not be presented here. Briefly, Doppler signals are processed primarily by spectral analysis, zero-crossing counter (ZCC) or time interval histogram (TIH). Spectral analysis by fast Fourier transform (FFT) gives more information about signal quality and composition than ZCC or TIH. Flow velocity determined from the maximal envelope of the FFT spectrum and from the ZCC compare favorably and are more reliable than TIH processing.¹²⁻¹⁴ Planimetered estimates of V_{\max} from FFT records must include the entire frequency envelope. ZCC signals are simpler to time average for determination of \bar{V} (mean velocity) from phasic V wave forms, but less information is apparent in the ZCC signal than in the spectral display.

Velocity Determination

Accurate velocity measurement requires appropriate instrument specifications, as discussed, as well as knowledge both of the character of the flow

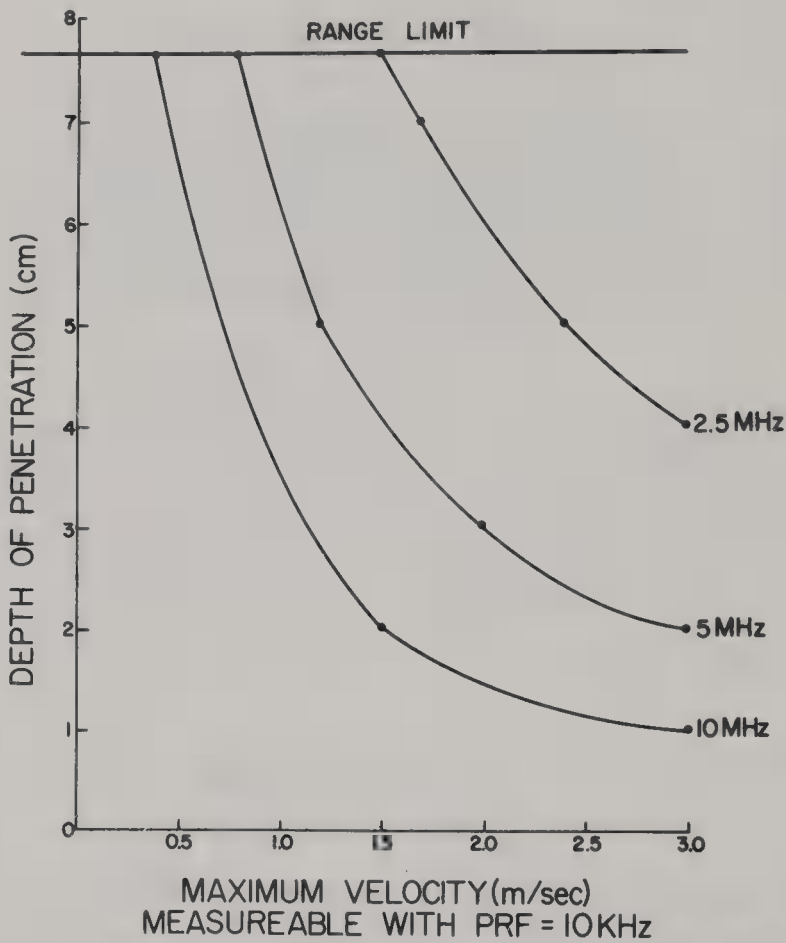


FIG 11. The effect of variation in transmission frequency on the penetration capability and maximum unambiguous velocity measurable by a pulsed Doppler system. (From Berman W, Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983. Used by permission.)

stream and the insonance angle, θ . Large vessels, with flat-flow profiles and laminar, nonturbulent flow must be sampled to assure that velocity information obtained within the sample volume (area of interrogation) reflects velocity across the entire flow stream. Small caliber vessels, areas near branch points, and turbulent flow streams (due to abnormal cardiac valves, intraluminal obstructions, or very high flow velocities) will have parabolic, distorted, or bidirectional flow profiles from which meaningful spatial mean velocity data cannot be obtained without interrogating the entire flow stream.³

Determination of the insonance angle, θ , is also critical. As the Doppler equation states,

$$V = (c \Delta f_o) / (2f_o \cos \theta),$$

where Δf is measured and V is calculated from $\cos \theta$. Figure 12 shows the relationship of $\cos \theta$ to the insonance angle. Between -15 degrees and $+15$ degrees, $\cos \theta$ is very close to 1. At higher insonance angles, $\cos \theta$ changes dramatically with small changes in θ (at $\theta = 70$ degrees, a 1-degree error in angle measurement causes a 6% error in V calculation). The angle θ should be minimized to reduce potential errors of this kind.

Flow Calculation

Flow velocity determination, even if made precisely, does not guarantee accurate flow "measurement." Vessel cross-sectional area is multiplied by \bar{V} to generate flow values. The measured variable in the vessel area determination is vessel diameter, d , a value squared in the area calculation (area = $\pi d^2/4$). Apparently, small errors in d measurement are magnified in flow calculations. If temporal mean velocity, \bar{V} , = 25 cm/sec, and $d = 0.9$ cm, $Q = 25 \times 0.634 \times 60 \text{ sec/minute} = 954 \text{ ml/minute}$; if $d = 1.0$ cm, $Q = 25 \times 0.785 \times 60 = 1,178 \text{ ml/minute}$, a 23% increase. Experience has shown that d must be measured each time \bar{V} is determined and at about the same time for results to be accurate.

Vessel diameter also is not a static value, but changes with the cardiac cycle. Systolic to diastolic variations of 10% to 15% are reported.¹⁵ By convention, systolic diameters are used, introducing a bias to overestimate

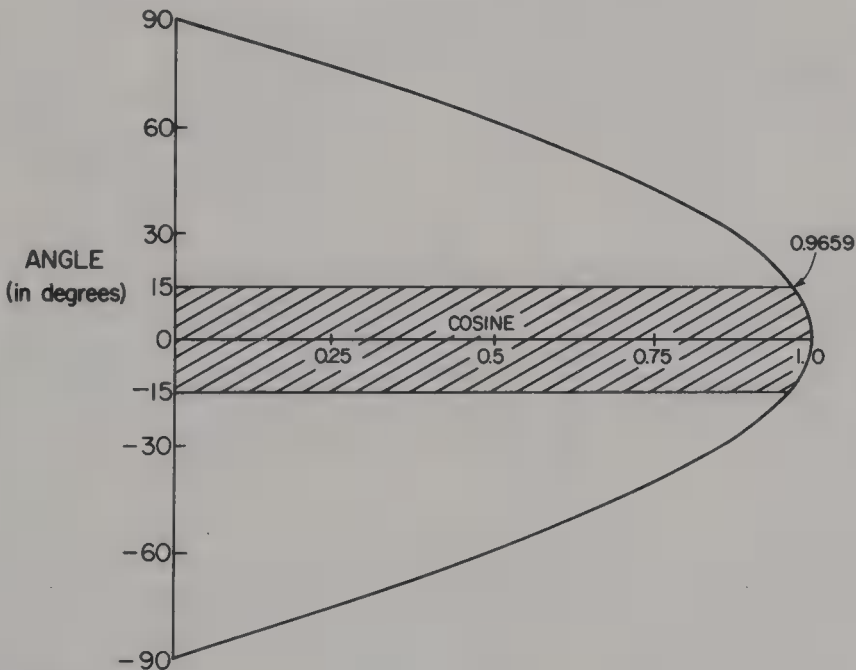


FIG 12.

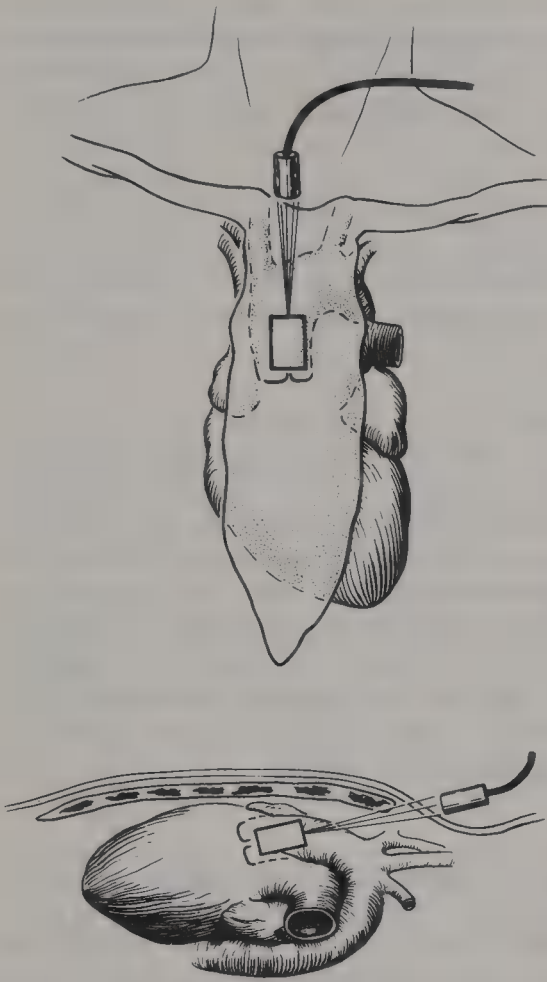
The relationship of the cosine of the angle of insonance to the angle itself. (From Berman W, Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983. Used by permission.)

flow from measured velocity. Temporal average of vessel diameter or, more ideally, instruments that track vessel diameter during velocity measurement are now being field tested as more accurate methods of true vessel area determination. In current practice, for calculation of ascending aortic flow, vessel diameter and flow velocity data are measured above the level of the aortic sinuses; this site excludes coronary flow considerations, a fact that tends to balance the effect of using the systolic vessel diameter for area computation.

Application

Noninvasive Determination of Cardiac Output and Great Vessel Flow Patterns

Interrogation of the aortic root from the suprasternal notch yields flow velocity signals of the ascending aortic flow stream. The suprasternal approach nearly guarantees a small (<15 degrees) insonance angle so that the $\cos \theta$ correction is minimal and need not be applied (Fig 13). Verification studies comparing the blind, suprasternal Doppler approach to calculations of aortic flow (cardiac output) measured according to the Fick principle¹⁶ or thermodilution cardiac output¹⁷ have shown excellent correlation of the ultrasound method with those accepted techniques. Loeber et al.¹⁸ have shown also that aortic flow calculations based on velocity sampling in the ascending aorta from the suprasternal notch compare very favorably with output determinations made with duplex scanning and θ quantification in the pulmonary trunk and tricuspid orifice. Sophisticated duplex studies of great vessel and intracardiac flow velocity indicate clearly the reliability and ease of the suprasternal notch approach when no intracardiac or great vessel shunts exist.¹⁹ Finally, interobserver and intraobserver variability studies, as well as the magnitude of physiologic variation, have been determined for adults^{20, 21} and children.²² Intraobserver variability in velocity ranged from 2% to 5%; interobserver variability from 3% to 6%; and physiologic variability in calculated flow on consecutive days of measurement from 10% to 20%. Intraobserver and interobserver peak velocity determinations are close; the variability may reflect small changes in the insonance angle, which is not measured in the blind, suprasternal approach, but may vary slightly from day to day. Flow variability on consecutive days of sampling may also reflect vessel diameter change with time. True biologic variation is no doubt responsible for some of the change as well. The suprasternal approach has been used now to quantify cardiac output measurements in the pediatric age group.²³⁻²⁵ Although values cited compare closely to numbers obtained invasively in earlier studies, several cautions must be emphasized. Because the sampling approach is blind, measurements may not be accurate when anatomy is dis

**FIG 13.**

Sampling the ascending aortic flow stream from the suprasternal approach. (From Berman W, Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983. Used by permission.)

torted (when the angle θ may be more than 15 degrees). Low or high flow states can distort the velocity profile and prohibit determination of a true mean spatial flow velocity. Patency of the ductus arteriosus will confuse cardiac output measurements by inclusion of the left-to-right shunt in the flow stream. And careful determination of the vessel diameter must accompany *each* flow velocity measurement, as changes in vessel size can occur quickly¹⁵ and affect flow calculations as noted previously.

Invasive Doppler-Based Cardiac Output Measurements

Doppler methodology can be used in systems that are invasive. Pulsed or continuous wave Doppler transducers applied directly to vessel walls can, with measurements of vessel diameter, be used to calculate flow data.^{26, 27} Moreover, Doppler transducers have been mounted on catheter tips and positioned within the flow stream.^{28, 29} The distortion of the flow stream that could occur consequent to an intravascular foreign body seems not to be problematic, but precise confirmation of the insonance angle is difficult

in this application. For relatively small vessels, with careful orientation of the catheter in the axis of the flow stream, useful quantitative data can be obtained. When the catheter position is unstable, if the orientation is not verifiable, or if the catheter position changes due to fling or migration, data interpretation may be impossible.

Regional Blood Flow

Regional flow velocity signals can be obtained with pulsed Doppler units in which the sample volume size and position are variable and controllable.

Qualitative Analysis

Flow velocity signals from peripheral vessels can be recorded and processed qualitatively by one of a number of variations on the "pulsatility index" method.³⁰⁻³² These angle-independent means of describing flow velocity patterns are ratios of peak systolic to trough diastolic flow velocity; absolute values for flow velocity and volume flow cannot be obtained accurately in most studies. These applications of wave form analysis can give useful information about distal vascular resistance, pulsatility of flow, and diastolic run off. Certain flow patterns have been associated with disease states, such as patent ductus arteriosus and intracranial hemorrhage.³³⁻³⁶ Still, absolute measurements, rather than dimensionless, referenced ratios remain the preferred approach to hemodynamic study.

Absolute Measurements

Doppler transducers can be applied directly, as noted previously,²⁷ to quantify regional flow patterns. A number of studies have shown that non-invasive methodology can be used to study some regional circulations. Greene et al.³⁷ used conventional duplex methodology to calculate renal blood flow, bilaterally, in humans. Flow measurements correlated with body surface area, and velocity patterns permitted blind identification of abnormal vessels in 11 of 11 cases, and normal vessels in six of seven instances. Greene and colleagues currently are using innovative hardware, gated and triggered to the electrocardiogram, in an effort to study coronary blood flow by the duplex method.³⁸ Uematsu et al.³⁹ have described a unique system for measurement of carotid blood flow. Inaccuracy due to variation in vessel size is minimized by an echo (A-mode) tracking device that samples a diameter measurement at 90 degrees to the vessel axis every 2 msec. That dimension is used to calculate flow from velocity measurements made by a separate three-transducer system using one emitting and two receiving crystals, at known angles to each other but not to the vessel. This four-transducer system quantifies vessel diameter throughout the cardiac cycle and permits calculation of θ (rather than measurement from a two-dimensional image) by virtue of two receiving crystals processing the same emitted/back-scattered signal. Correlation of measurements with a simulated system was excellent, as was reproducibility of flow de-

terminations within the same recording session (3.8%) or on consecutive sessions (10.6%). A similar diameter tracking device has been used by Marsal et al. to measure aortic flow velocity in the aorta of the human fetus.⁴⁰⁻⁴² Imaging and sampling from the subdiaphragmatic aortic flow stream, flow calculation per estimated fetal weight has given values somewhat less than, but comparable to, measurements of umbilical blood flow in the sheep fetus.^{43, 44} Gestational flow patterns from a number of laboratories have confirmed relative stability of flow/weight over the last third of gestation,^{45, 46} an increase in vessel diameter without change in mean spatial flow velocity as the mechanism of flow increase with time, and the association of abnormal flow velocity patterns and calculated flow with a number of disease states.⁴⁷⁻⁵¹

Assessment of Shunts

The pioneering studies of Goldberg and Allen et al. have shown the usefulness of pulsed Doppler devices in quantifying abnormal pulmonary to systemic blood flow ratios due to intracardiac shunts. Although flow turbulence in some malformations can complicate sampling, and although determination of the insonance angle in the pulmonary artery is difficult due to its anterior/posterior angulation (an unmeasured azimuthal angle), correlations of pulmonary and systemic flows, as well as the flow ratio, have been close in the clinical setting as well as in the animal laboratory.^{52, 53}

Other Applications of Doppler Flowmetry

Directional Flow Velocity Determination

Doppler systems quantify the direction of the flow stream. In certain applications, such as assessment of congenital heart disease,⁵⁴ rheumatic fever, the premature with a patent ductus arteriosus, or the postoperative cardiac patient with an aortopulmonary shunt, useful information is obtained by determining the direction of flow during all or a portion of the cardiac cycle. An example is the diastolic backflow in the aorta detectable after a successful aortopulmonary shunt.⁵⁵ Conventional or digital multigated color-coded images can be used in this application.

Estimation of Pulmonary Artery Pressure

A number of methods have been described that reference systemic flow velocity to systemic systolic blood pressure, then quantify velocity gradients between cardiac chambers due to intracardiac defects⁵⁶ or tricuspid incompetence,⁵⁷ and back-calculate right ventricular systolic pressure from the measured data. In the absence of right ventricular outflow tract obstruction, this value may be taken to equal systolic pulmonary artery pressure. In experienced and meticulous hands, this approach has generated values close to those measured directly at catheterization.

A second approach uses analysis of the pulmonary artery velocity wave form to estimate pressure. Studies have shown an inverse relationship between systolic pressure and the rate of rise of the velocity tracing. This finding confirms and parallels the demonstration of a direct relationship between right ventricular dP/dt and right ventricular systolic pressure in normally contracting hearts.⁵⁸ Therefore, the time to peak flow (acceleration time), as an absolute number or referenced to right ventricular ejection time, has been proposed as an index of pulmonary artery pressure or vascular resistance. Initial studies have been promising and the correlations high; the deviation of points from the regression lines, however, has been substantial, and the limits of confidence broad.⁵⁹⁻⁶¹

Assessment of Hemodynamic Function

As both acceleration time and dP/dt have been shown to correlate with ventricular systolic pressure, it is not surprising that velocity and acceleration intervals have been used to assess "contractility" by approximating dP/dt . Initial studies using Doppler methodology have shown a strong afterload dependence of peak velocity, but relative afterload independence of time to peak velocity (acceleration time).^{29, 58-63} Subsequent studies in dogs have shown a strong negative correlation between acceleration time and left ventricular dP/dt over a wide range of inotropic and loading conditions. The prospect for noninvasive approximation of the contractile state awaits completion of pilot studies in which this methodology is applied to controlled clinical evaluations.

References

1. Schwartz MD, De Cristofaro D: Review and evaluation of range-gated, pulsed, echo-Doppler. *J Clin Eng* 1978; 3:153.
2. Baker DW: Application of pulsed Doppler techniques. *Radiol Clin N Am* 1980; 18:79.
3. Eldridge MW, Alverson DC, Howard EA, et al: Pulsed Doppler ultrasound: Principles and instrumentation, in Berman W Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983, pp 5-40.
4. Hatle L, Angelsen B: Doppler Ultrasound in Cardiology. Philadelphia, Lea & Febiger, 1982, p 32.
5. Shung KK, Sigelmann RA, Reid JM: Scattering of ultrasound by blood. *IEEE Trans Biomed Engl BME* 1976; 23:460.
6. Stevenson JG, Kawabori I: Noninvasive determination of pressure gradients in children: Two methods employing pulsed Doppler echocardiography. *JACC* 1984; 3:179.
7. Kosturakis D, Allen HD, Goldberg SJ, et al: Noninvasive quantification of stenotic semilunar valve areas by Doppler echocardiography. *JACC* 1984; 3:1256.
8. Currie PJ, Hagler DJ, Seward JB, et al: Instantaneous pressure gradient: A simultaneous Doppler and dual catheter correlative study. *JACC* 1986; 7:800.

9. Come PC: The optimal Doppler examination: Pulsed, continuous wave or both? *JACC* 1986; 7:886.
10. American Institute of Ultrasound in Medicine, Bioeffects Committee: *Safety Considerations for Diagnostic Ultrasound*. Bethesda, AIUM publication no. 316, 1984.
11. Jenkins GM, Watt DC: Spectral analysis. London, Holden Day, 1969.
12. Voyles WF, Altobelli SA, Fisher DC, et al: A comparison of digital and analog methods of Doppler spectral analysis for quantitation of flow. *Ultrasound Med Biol* 1985; 11:727.
13. Lunt MJ: Accuracy and limitations of the ultrasonic Doppler blood velocimeter and zero crossing detector. *Ultrasound Med Biol* 1975; 2:1.
14. Burckhardt CB: Comparison between spectrum and time interval histogram of ultrasound Doppler signals. *Ultrasound Med Biol* 1981; 7:79.
15. Alverson DC, Eldridge MW, Johnson JD, et al: Noninvasive measurement of cardiac output in healthy preterm and term newborn infants. *Am J Perinat* 1984; 1:148.
16. Alverson DC, Eldridge M, Dillon T, et al: Noninvasive pulsed Doppler determination of cardiac output in neonates and children. *J Pediatr* 1982; 101:46.
17. Huntsman LL, Steward DK, Barnes SR, et al: Noninvasive Doppler determination of cardiac output in man. *Circulation* 1983; 67:593.
18. Loeber CP, Goldberg SJ, Allen HD: Doppler echocardiographic comparison to flows distal to the four cardiac valves. *JACC* 1984; 4:268.
19. Grenadier E, Lima CO, Allen HD, et al: Normal intracardiac and great vessel Doppler flow velocities in infants and children. *JACC* 1984; 4:343.
20. Gardin JM, Dabestani A, Matin K, et al: Reproducibility of Doppler aortic blood flow measurements: Studies on intraobserver, interobserver and day-to-day variability in normal subjects. *Am J Cardiol* 1984; 54:1092.
21. Voyles WF, Greene ER, Miranda IP, et al: Observer variability in serial non-invasive measurements of stroke index using pulsed Doppler flowmetry. *ISA Biomed Sci Instru* 1982; 18:67.
22. Claflin KS, Alverson KC, Pathek D, et al: Cardiac output in the newborn: Variability of the pulsed Doppler method, abstract. *Pediatr Res* 1986; 20:1240.
23. Walther FJ, Siassi B, Ramadan NA, et al: Pulsed Doppler determinations of cardiac output in neonates: Normal standards for clinical use. *Pediatrics* 1985; 76:829.
24. Lees MH: Cardiac output determination in the neonate. *J Pediatr* 1983; 102:709.
25. Alverson DC, Aldrich M, Angelus P, et al: Longitudinal trends in left ventricular cardiac output in healthy infants over the first year of life, abstract. *Pediatr Res* 1985; 19:228.
26. Steingart RM, Meller J, Barovick J, et al: Pulsed Doppler echocardiographic measurement of beat-to-beat changes in stroke volume in dogs. *Circulation* 1980; 62:542-548.
27. Hartley CJ, Cole JS: An ultrasonic pulsed Doppler system for measuring blood flow in small vessels. *J Appl Physiol* 1974; 37:28.
28. Cole JS, Hartley CJ: The pulsed Doppler coronary artery catheter. *Circulation* 1977; 56:18.
29. Berman W Jr, Alverson DC: Assessment of hemodynamic function with pulsed Doppler ultrasound. *JACC* 1985; 5:104S.

30. Bejar R, Manning F: Pulsatility index, patent ductus arteriosus, and brain damage. *Pediatrics* 1982; 69:818.
31. Badda H, Hajjar W, Chua C, et al: Noninvasive diagnosis of neonatal asphyxia and intraventricular hemorrhage by Doppler ultrasound. *J Pediatr* 1979; 95:775.
32. Volpe JJ, Perlman JM, Hill A, et al: Cerebral blood flow velocity in the human newborn: The value of its determination. *Pediatrics* 1982; 70:147.
33. Snider AR, Howard EA: The evaluation of cerebral artery flow patterns with Doppler ultrasonography, in Berman W Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983, pp 93–140.
34. Eldridge M, Berman W Jr, Green ER, et al: Femoral blood flow in term and premature newborn infants. *J Ultrasound Med* 1984; 3:53.
35. Alverson DC, Eldridge M, Aldrich M, et al: Effect of patent ductus arteriosus on lower extremity blood flow velocity patterns in preterm infants. *Am J Perinatol* 1984; 1:216.
36. Wilcox WD, Carrigan TA, Dooley KJ, et al: Range-gated pulsed Doppler ultrasonographic evaluation of carotid arterial blood flow in small preterm infants with patent ductus arteriosus. *J Pediatr* 1983; 102:294.
37. Greene ER, Venters MD, Avasthi PS, et al: Noninvasive characterization of renal artery blood flow. *Kidney Internatl* 1981; 20:523.
38. Greene ER: Unpublished observations, 1986.
39. Uematsu S, Yang A, Preziosi TJ, et al: Measurement of carotid blood flow in man and its clinical application. *Stroke* 1983; 14:256.
40. Eik-Ness SH, Marsal K, Brubakk AO, et al: Ultrasonic measurement of human fetal blood flow. *J Biomed Eng* 1982; 4:28.
41. Lindblad A, Marsal K, Vernersson E, et al: Fetal circulation during epidural analgesia for caesarean section. *Br Med J* 1984; 288:1329.
42. Marsal K, Lindblad A, Lingman G, et al: Blood flow in the fetal descending aorta: Intrinsic factors affecting fetal blood flow, i.e., fetal breathing movements and cardiac arrhythmia. *Ultrasound Med Biol* 1984; 10:339.
43. Berman W Jr, Goodlin RC, Heymann MA, et al: The measurement of umbilical blood flow in fetal lambs in utero. *J Appl Physiol* 1975; 39:1056.
44. Rudolph AM, Heymann MA: The fetal circulation. *Ann Rev Med* 1968; 19:195.
45. Eldridge MW, Berman W Jr, Greene ER, et al: Serial Doppler ultrasound measurements of human fetal abdominal aortic blood flow. *J Ultrasound Med* 1985; 4:453.
46. Erskine RLA, Ritchie JWK: Quantitative measurement of fetal blood flow using Doppler ultrasound. *Br J Obstet Gynaecol* 1985; 92:600.
47. Lingman G, Dahlstrom JA, Eik-Nes SH, et al: Haemodynamic assessment of fetal heart arrhythmias. *Br J Obstet Gynaecol* 1984; 91:647.
48. Lingman G, Marsal K: Circulatory effects of fetal heart arrhythmia, in Lingman G (ed): *Human Fetal Haemodynamics*. Sweden, Malmo, 1985, pp VII–VI20.
49. Lingman G, Lundstrom NR, Marsal K, et al: Fetal cardiac arrhythmia: Clinical outcome of 113 cases, in Lingman G (ed): *Human Fetal Haemodynamics*. Sweden, Malmo, 1985, pp VII1–VII13.
50. Eriksen PS, Marsal K: Acute effects of maternal smoking on fetal blood flow. *Acta Obstet Gynecol Scand* 1984; 63:391.
51. Eldridge MV, Berman W Jr: Serial measurement of human fetal aortic blood flow, in Berman W Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983, pp 211–224.

52. Meijboom EJ, Valdes-Cruz LM, Horowitz S, et al: A two-dimensional Doppler echocardiographic method for calculation of pulmonary and systemic blood flow in a canine model with a variable-sized left-to-right extracardiac shunt. *Circulation* 1983; 68:437-445.
53. Valdes-Cruz LM, Horowitz S, Mesel E, et al: A pulsed Doppler echocardiographic method for calculation of pulmonary and systemic flow: Accuracy in a canine model with ventricular septal defect. *Circulation* 1983; 68:597.
54. Stevenson JG: Pulsed Doppler characterization of intracardiac flow patterns, in Berman W Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983, pp 141-170.
55. Stevenson JG, Kawabori I, Bailey WW: Noninvasive evaluation of Blalock-Taussig shunts: Determination of patency and differentiation from patent ductus arteriosus by Doppler echocardiography. *Am Heart J* 1983; 106:1121.
56. Marx GR, Allen HD, Goldberg SJ: Doppler echocardiographic estimation of systolic pulmonary artery pressure in pediatric patients with interventricular communications. *JACC* 1985; 6:1132.
57. Curie PJ, Seward JB, Chan K-L, et al: Continuous wave Doppler determination of right ventricular pressure: A simultaneous Doppler-catheterization study in 127 patients. *JACC* 1985; 6:750.
58. Yabek SM, Berman W Jr, Dillon T: Right ventricular function in children with congenital heart disease. *Am J Cardiol* 1984; 53:899.
59. Kitabatake A, Inoue M, Asao M, et al: Noninvasive evaluation of pulmonary hypertension by a pulsed Doppler technique. *Circulation* 1983; 68:302.
60. Kosturakis D, Goldberg SJ, Allen HD, et al: Doppler echocardiographic prediction of pulmonary arterial hypertension in congenital heart disease. *Am J Cardiol* 1984; 53:1110.
61. Martin-Duran R, Larman M, Trugeda A, et al: Comparison of Doppler-determined elevated pulmonary arterial pressure with pressure measured at cardiac catheterization. *Am J Cardiol* 1986; 57:859.
62. Alverson DC, Berman W Jr: Noninvasive assessment of myocardial contractility with pulsed Doppler ultrasound, in Berman W Jr (ed): *Pulsed Doppler Ultrasound in Clinical Pediatrics*. Mt Kisco, NY, Futura Publishing Co, 1983, pp 197-210.
63. Alverson DC, Berman W Jr, Blomquist T, et al: Assessment of myocardial contractility using pulsed Doppler ultrasound, abstract. *Pediatr Res* 1984; 18:132.

Advances in Understanding the Pathogenesis of Persistent Diarrhea in Young Children

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Chronic persistent diarrhea is a frequent problem encountered in pediatrics, especially in infants and young children. This type of diarrhea may be secondary to simple dietary indiscretion, e.g., excessive fluid intake, or to a more difficult to solve diagnostic problem such as cow's milk protein enteropathy or *Giardia lamblia* infection in a child attending a day-care center (DCC). Taken altogether, the problem of chronic diarrhea imposes a moderate burden on families and society. For many pediatric gastroenterologists, this entity probably accounts for almost 30% of outpatient referrals. Pediatricians evaluate children with this symptom on a regular basis. This review emphasizes the nonspecific and "more specific" causes of diarrhea in young children and their underlying pathogenesis.

Normal Physiology and Pathophysiology

Diarrhea (Gr. *dia*, through, plus *rhein*, to flow), defined as the frequent passage of watery stools, is a symptom for which an underlying abnormality should always be sought. This is most important for the growing child so that treatment may be as specific as possible. The alimentary tract receives, mixes, digests, and absorbs variable amounts of exogenous and endogenous nutrients, fluids, and electrolytes leading to the excretion of a small volume of stool. In the adult and older child, daily fluid fluxes include approximately 2 L of ingested liquids and an additional 6 to 7 L of salivary, gastric, hepatobiliary, pancreatic, and intestinal fluid.¹ Since only 100 to 200 ml of water is normally present in stool each day, greater than 95% of this large volume of fluid is efficiently reabsorbed. The small intestine absorbs water at a rate of 200 to 400 ml/hour.² Although the small gut is primarily responsible for absorption, the colon is capable of significantly

increasing this process such that water absorption by both small and large intestine can be approximately 500 ml/hour.³ Above this rate, excess water is excreted into the stool. If this concept of absorption rate is thought of with respect to decreased absorptive surface area (or enterocyte damage), the potential for accentuated malabsorption of water can be easily understood. Other pathophysiologic mechanisms responsible for diarrhea include osmotic retardation of water absorption, increased secretion of water and electrolytes, abnormal intestinal motility, and bacterial overgrowth of the small intestine.

Chronic Nonspecific Diarrhea

Definition and Diagnosis

Chronic nonspecific diarrhea, in otherwise well children, is not a single clinical entity; there are multiple causes for this common problem, which usually occurs between the ages of 6 and 39 months. It is distinguished from more serious diarrheal illness by lack of evidence for malabsorption, failure-to-thrive, or dehydration.^{4, 5} Because the children in the original report responded to antimicrobial therapy, bacterial overgrowth was a possible cause of the diarrhea.⁴ Davidson and Wasserman⁵ described a group of children ingesting low-fat diets, but dietary manipulation, except for the avoidance of cold or hyperosmolar beverages, was of no treatment benefit. Underlying small intestinal dysmotility, an unproven cause of nonspecific diarrhea, could potentially be unmasked by ingestion of cold (or ice) and hyperosmolar fluids. However, disordered small intestinal motility was recently reported in some children with nonspecific diarrhea and is discussed later.⁶ Frequently, the chronic process in these children is preceded by acute diarrhea leading to multiple dietary manipulations including excessive clear liquids (high carbohydrate) and restricted milk (low fat) intake. The diagnosis of chronic nonspecific diarrhea is made by exclusion with special emphasis on the dietary history and a screening stool examination (pH, reducing substances, fat, red and white blood cells).

Possible Causes

Overfeeding

In an important outpatient study,⁷ two groups of children were evaluated by quantitation of fecal output, dietary energy-protein intake, and nonprotein fluid intake. Their fluid intakes were highly different: group A, 196 ml/kg/day, and group B, 91 ml/kg/day. The group A nonprotein fluid intake was decreased to 90 ml/kg/day with no other change in their diet. Evaluation at 2 weeks and again at 6 to 8 weeks showed a significant decrease in stool frequency and improvement in stool consistency. The children in

group B showed no change in stool patterns. Three patients in group A were consuming large volumes of water alone, indicating that high osmolality may not be an important cause of diarrhea in many patients. The authors concluded that the absorptive capacity of the intestinal tract can be exceeded by intakes greater than 200 ml/kg/day and that careful attention should be given to the volume of intake as well as the type of fluid.

Decrease in Fat Intake

A retrospective study⁸ divided patients with chronic nonspecific diarrhea into four groups: I, low fat (27% of total calories); II, low fat and marginal calories; and III and IV, normal intakes. Daily fat consumption was increased to 4.5 to 6.0 gm/kg/day in the first three groups, and this dietary change was associated with resolution of frequent stools. The authors stated that dietary carbohydrate, fiber, and caloric content did not appear to have as significant a role as fat intake (at least 4 gm/kg/day). Unfortunately, the test diets of their patients were not isocaloric to the original intake. Hence, the improvement may have resulted, at least in part, from a concomitant decrease in carbohydrate and fiber. The mechanism responsible for improvement after ingestion of increased fat was not clear, but probably related to slowing of intestinal motility and delay in gastric emptying. Different results were noted in a more recent prospective study⁹ to determine the effect of basal fat consumption (either 25% or 50% of total calories as fat) on nonspecific diarrhea in a small group of children who were evaluated *after* an observation period *prior* to the initiation of dietary changes. The authors discovered no association between low-fat diets and chronic nonspecific diarrhea because of their inability to find a significantly low-fat intake prior to initiating dietary intervention. This spontaneous resolution of the diarrhea precluded an assessment of the effect of altering fat intake on stool frequency.

Unsuspected Carbohydrate Malabsorption

Significant loss of absorptive surface area may occur after viral gastroenteritis with consequent impairment of disaccharidase activity, especially lactase. Secondary lactose malabsorption may persist for many months following acute infectious enteritis.¹⁰ Osmotic diarrhea occurs because the undigested carbohydrate stimulates secretion of large volumes of luminal water with subsequent gut distention and increased intestinal motility. Colonic bacterial fermentation of undigested sugar accentuates this osmotic trapping of water. A fecal pH (<6.0) detects the presence of fermentation products and a stool reducing sugar of 0.5% indicates carbohydrate malabsorption. The breath hydrogen test is the most accurate noninvasive study to identify a more subtle sugar absorption problem.¹¹

Congenital sucrase-isomaltase deficiency¹² has not been reported in children with chronic nonspecific diarrhea. In a recent large study, 75% of 31 Greenlandic children with sucrose malabsorption were reported to be malnourished, and many were dehydrated at admission to hospital.¹³

A few young infants may not be able to handle glucose polymers during recovery from acute enteritis, which is more persistent and ultimately becomes more chronic. Since glucose polymers are now the major source of carbohydrate in semielemental formulas (i.e., Pregestimil), chronic diarrhea may become a more frequently recognized problem. In contrast to pancreatic amylase, which is reduced in early infancy, mucosal glucoamylase is present in relatively high concentrations even in premature infants.¹⁴ The glucose polymers are at least 50% short-chain oligosaccharides that are hydrolyzed by mucosal glucoamylase; they also consist of long-chain oligosaccharides that require pancreatic amylase for digestion. Hence, intestinal mucosal injury in early infancy could potentially lead to glucose polymer (e.g., corn syrup solids) intolerance and persistent diarrhea. A malnourished infant has been described with documented mucosal injury, pancreatic amylase deficiency, and suspected (but unproven) glucoamylase deficiency.¹⁵

Complex carbohydrates may be incompletely digested and absorbed even by a normal small bowel; the carbohydrates present in wheat, oat, and rice flour are documented examples.¹⁶ Sorbitol ingestion may be another cause of unexplained diarrhea in infants and young children. This is a sweet-tasting nonreducing sugar that is used as an "inert ingredient" in some medication¹⁷ and as a sugar substitute in certain candies and "sugarless" gums,¹⁸ and it is found in high concentrations in processed apple juice. One piece of gum may contain as much as 2 gm sorbitol, a sugar that is poorly digested in the small intestine and finally metabolized to fructose. Activated charcoal is commercially available suspended in sorbitol, which improves its palatability and acts as a cathartic.¹⁷ A 70% sorbitol solution provides approximately 110 gm sorbitol per 150 ml (30 gm charcoal) packaged container with an osmolality of greater than 1,000 mOsm/L. Charcoal preparations with any concentration of sorbitol should be used with caution in infants.

Chronic "More Specific" Diarrhea

Acute diarrheal disease is a common occurrence among infants and young children. The acute episode resulting from viral and bacterial infections is usually self-limited; however, some children have persistence of diarrhea or a postgastroenteritis syndrome. This syndrome is not well understood; it certainly is related to well-documented, regularly occurring lactose intolerance. Furthermore, transient protein intolerance, intestinal dysmotility, and small bowel bacterial overgrowth may be additional factors responsible for the pathogenesis of persistent diarrhea in some of these otherwise healthy children. Allergic gastroenteropathy, iatrogenic diarrhea, and laxative abuse are also reviewed as "more specific" causes for persistent diarrhea in young children.

Postgastroenteritis Syndrome

Transient Protein Intolerance

There may be a relationship between severity of intestinal epithelial cell damage, existence of protein intolerance, and occurrence of carbohydrate intolerance. The permeability of the intestine has been studied with simultaneous use of two low-molecular-weight sugars, lactulose, and rhamnose.¹⁹ These sugars differ in their mucosal permeation. Lactulose resists intestinal hydrolase activity and is not absorbed by normal enterocytes, whereas rhamnose normally permeates the intact intestinal epithelial cell more readily.¹⁹ After analysis of lactulose/rhamnose excretion ratios, children with acute gastroenteritis had a greatly increased permeability. Children retested 3 to 16 weeks after complete recovery from their acute disease had normal permeability; in contrast, children with chronic diarrhea also had increased permeability, but significantly less than children in the acute gastroenteritis group. The authors concluded that intestinal sugar permeability is a reliable and useful index of mucosal integrity in that abnormal proximal small intestinal morphology was associated with increased permeability.

In an earlier study, cow's milk protein was shown to have a deleterious effect on the small intestinal mucosa of infants recovering from acute enteritis.²⁰ Because these patients had acquired carbohydrate intolerance, it was suggested that the management of infants with acquired sugar malabsorption should include temporary dietary exclusion of the offending sugar and protein. Also, it was speculated that acute infectious enteritis may play a role in the genesis of cow's milk protein enteropathy.

Intestinal Dysmotility

Undigested carbohydrates cause large volumes of water to enter the intestinal lumen. In addition to enterocyte damage, fluid distention of the bowel stimulates more active intestinal motility and consequent shortening of transit time. This increase of bowel activity has always been considered an important defense mechanism; however, more recent recognition of disordered small intestinal motility patterns may eventually lead to modification of this generally accepted concept. Upper small intestinal motility was studied in children with suspected intestinal pathology (but subsequently discovered to be normal) and proven gastrointestinal disease and in those with "toddler" diarrhea (chronic nonspecific diarrhea).⁶ Migrating motor complexes (MMCs) were seen as bands of high amplitude rhythmic pressure waves that propagated from duodenum to the jejunum. The authors tested the ability of intravenous cholecystokinin or intraduodenal dextrose to disrupt the MMC activity front in these children. Disruption of MMCs by eating is well described, and their major function may be removal of intestinal contents following absorption of nutrients.²¹ Cholecystokinin has been considered one of the candidate gut hormones responsible for the change

in fasting to the postprandial pattern of motility.²² The characteristics of the MMCs in the three groups of children did not differ except for their response to dextrose.⁶ Major differences were noted between children with nonspecific toddler diarrhea and controls. The entire group with nonspecific diarrhea failed to normally disrupt their MMCs in response to duodenal dextrose (food was not tested). The reasons for this failure were unclear, but it was speculated that this lack of MMC disruption may indicate a generalized dysmotility pattern. Failure of normal intestinal motor responses may prove to play a major role in the pathogenesis of nonspecific (and more specific) diarrhea.

Small Intestinal Bacterial Overgrowth

The small intestine proximal to the distal ileum is normally sparsely populated with bacteria. The normal flora of the upper small bowel includes no more than 10^4 organisms/ml of duodenal or jejunal aspirate. These organisms are primarily gram positive staphylococci, streptococci, lactobacilli, and some fungi. Aerobic coliforms and anaerobes are almost never demonstrated in the absence of an anatomic lesion or motility disorder of the intestine. In addition, the presence of unabsorbed carbohydrates in the gut lumen enhances the proliferation of bacteria.²³ Bacterial proliferation may be especially prominent in children with acute diarrheal disease.²⁴⁻²⁶ The significance of increased bacterial numbers and types of organisms, including *Escherichia coli*, is not certain. It is clear, however, that the intestinal bacterial flora can be easily disturbed.²⁴ More common enteropathogens such as *Shigella* and *Salmonella* are also found in the upper small bowel during acute disease.²⁷ *Bacteroides* species have been identified in significant concentrations in normal children with acute diarrhea.²⁶ In this latter study, no differences were found in the duodenal microflora to help distinguish infants with more chronic diarrhea (greater than 7 days in the hospital) from those who experienced an earlier resolution of the diarrhea.

The diagnosis of small intestinal bacterial overgrowth is usually by culture of abnormal bacteria in the duodenal or jejunal fluid. In a more recent study, unsuspected bacterial contamination of the small bowel was diagnosed in children with chronic diarrhea by the noninvasive method of breath hydrogen (H_2) testing.²⁸ Significant concentrations of *Pseudomonas* and *Bacteroides* were cultured from duodenal aspirates. The patients were lactose intolerant with sucrose intolerance noted in 50% of the patients. Deconjugated bile acids were not detected in the duodenal fluid of any patient. In children with positive duodenal aspirate cultures, the fasting breath H_2 level was elevated; this was followed after 1 hour by a sustained rise in H_2 as unabsorbed carbohydrate reached the colon. The authors suggested that these findings may be useful for detecting the presence of bacterial overgrowth.²⁸ As indicated by the breath H_2 results, dietary modification with lactose and sucrose withdrawal was the initial treatment in all appropriate patients. When dietary restriction alone did not cause cessation of symptoms, all patients responded to oral antibiotic ther-

apy. The interesting findings of this study should heighten suspicion of bacterial overgrowth in infants and young children with chronic diarrhea, especially those who have been unresponsive to elimination of dietary carbohydrates.

An elegant scanning electron microscopy (EM) study by Poley²⁹ adds further credence to the concept of bacterial overgrowth in young children with chronic diarrhea. The following EM findings were noted in the vicinity of microorganisms: (1) breaks in the surface coat of microvilli, (2) loss of glycocalyx, and (3) rare mucosal microulcerations. Whether abnormal small intestinal bacteria play any significant role in the pathogenesis of persistent diarrhea in children remains to be determined. Furthermore, the pathophysiologic consequences of bacterial overgrowth are also uncertain, such as (1) stimulation of fecal water and electrolytes by secretagogues, e.g., deconjugated bile acids and hydroxy fatty acids, (2) deconjugation of bile salts potentially leading to fat malabsorption via decreased micellar solubilization, and (3) vitamin B12 malabsorption.

Allergic Gastroenteropathy

The manifestations of cow's milk allergy are protean and usually occur before 6 months of age. The diarrhea may be mild and nonspecific, but it tends to be more intractable, which usually refers to a patient with malnutrition and failure-to-thrive. Children sometimes develop anemia with blood in stools, edema, and protein-losing enteropathy³⁰; or diarrhea with pneumonitis, pulmonary hemosiderosis, and iron deficiency.³¹ In one study of children with recurrent nonspecific diarrhea, there was a high incidence of food allergy.³² (This is discussed in detail in the chapter by Lemanske and in references 33 to 48.)

Iatrogenic Diarrhea

The most notable culprits in iatrogenic diarrhea are drugs. Antibiotics frequently cause simple acute diarrhea, but the process may become chronic because of disruption of the normal intestinal bacterial flora and the potential for emergence of resistant organisms or bacterial overgrowth. In childhood, even the more severe problems of pseudomembranous colitis can present as mild, chronic diarrhea.⁴⁹ It may be caused by any antibiotic, but clindamycin and ampicillin have been most frequently associated with this entity. The discovery of fecal leukocytes should prompt a sigmoidoscopic examination to look for typical pseudomembranes in any child whose diagnosis is considered in doubt.⁴⁹ Lactulose and laxatives induce diarrhea as part of their therapeutic action; a dose-dependent side effect of magnesium-containing antacids is diarrhea. A careful history of drug ingestion should be taken of every patient with diarrhea and the offending agent discontinued if at all possible. Child care providers should also be precise about "clear liquid" recommendations for their patients with diarrhea. This

is especially important in terms of hyperosmolar fluids such as pineapple juice (900 mOsm/L) and apple juice (650 mOsm/L).

Laxative Abuse (Munchausen's Syndrome By Proxy)

Surreptitious laxative abuse should always be considered as a possible cause for chronic diarrhea in the child without a clear explanation for the loose motions.⁵⁰ The patient is usually healthy appearing; the parent is always helpful and grateful—perhaps almost too willing to accept the child's symptoms. The diagnosis of Munchausen's syndrome by proxy is one of exclusion and requires a high index of suspicion. Detecting the presence of phenolphthalein, an ingredient in many laxatives, is easily accomplished by alkalization of a stool sample (turns pink) with sodium hydroxide. A urine screen may detect metabolites of laxatives that do not contain phenolphthalein. Management is always difficult and requires psychiatric consultation.

Day-Care Center Diarrhea

Families have changed: more mothers are working and more young children are being cared for outside the home. Infants and young children make up an increasing percentage of the estimated 2 million children attending DCCs. These centers should be recognized as special epidemiologic environments because they have a unique set of risk factors promoting disease transmission.^{51, 52} Pediatricians, social scientists, and health policy makers have been increasingly concerned about mounting evidence that DCCs significantly impact on the frequency of infectious illnesses not only in children, but also in day-care staff and household contacts.

Epidemiology

Enteric infections represent the largest and most well-defined infectious disease problem in DCCs. The most frequent modes of spread are the fecal-oral route (person to person) and fomite contamination, e.g., toys. Food-borne and waterborne spread is less common. Features of DCC infections include the following⁵³: (1) all organisms cause discrete outbreaks that may last for months; (2) diseases are spread most easily among young children (less than 3 years of age) because of diapers, lack of toilet training, and high frequency of oral activity; and (3) prevention depends on maintenance of maximal hygienic standards.

Enteric Infections in Day-Care Centers

The first large report was a 19-month prospective study of children attending 20 DCCs in Houston.⁵⁴ Nine centers had 15 outbreaks of diarrhea; an enteropathogen was identified in all outbreaks. *Shigella* was detected in

five, rotavirus in two, *Giardia* in one, and in the remaining seven multiple pathogens were identified. Rotavirus and *Giardia* occurred only in children less than 3 years of age. The family member secondary attack rate was 11% in six centers; the organisms were *Shigella*, 26%; rotavirus, 15%; and *Giardia*, 17%. The message was clear, that is, significant spread does occur from preschool children to the community at large. Observations of practical significance that emerged from this study were the following: (1) for all outbreaks, children with diarrhea continued to attend the DCC; (2) isolation rooms for ill children were generally not available; (3) teachers seldom washed their hands between infants; and (4) parents would often deny that their children were symptomatic.⁵⁴

To clarify the prevalence, clinical significance, and epidemiology of intestinal parasites in DCCs, a survey of 22 centers in Toronto was attempted.⁵⁵ There were between 17 and 140 children in the centers surveyed; the children's staff and families completed a questionnaire and submitted stool samples for testing. A total of 900 children and 146 staff participated; overall, intestinal parasites were identified in 19% of the children and 14% of the staff. *Giardia* and *Dientamoeba fragilis* accounted for most of the parasites detected. Dientamebiasis was associated with cat ownership. Symptoms were not correlated with infection; approximately 20% of the uninfected and those with the aforementioned parasites were asymptomatic. It was suggested that drug therapy should be reserved for symptomatic patients and perhaps asymptomatic food handlers.⁵⁵

Another prospective study was a 2-year assessment of diarrheal illness in infants and young children in 22 DCCs in Arizona.⁵⁶ In 7,464 child-months of observation, 465 sporadic cases and 170 outbreak-associated cases were identified (18 outbreaks in 2 years). Pathogens were discovered in 20% of the diarrheal episodes, mainly rotavirus, *Giardia*, and *Campylobacter*. As noted in previous studies, giardiasis was more common in toddlers and noted in 19% of asymptomatic child contacts. The rotavirus infection rate was significantly higher in infants than in toddlers. The findings also suggested that children in DCCs have a significantly higher rate of diarrheal illness than do those cared for in the smaller group setting of day-care homes (with four to fewer children) or at home.⁵⁷ Like the Houston study,⁵⁴ it was found that the rate of diarrhea in DCCs was lower in the second year of observation. The continuing contact with health personnel probably increased awareness of disease control in the centers, especially better hygiene such as child and staff hand-washing.

Unlike *Shigella*,^{54, 55} *Salmonella*⁵⁸ infections have rarely been documented in DCC outbreaks. There has been one reported outbreak of enteropathogenic *E. coli*-induced diarrhea in an infant and toddler center.⁵⁹ *E. coli* may be a more important cause of diarrhea in DCCs than is generally recognized because this enteropathogenic organism is not routinely sought by most laboratories. An outbreak of *Cryptosporidium*-induced diarrhea has been noted in a DCC⁶⁰ in immunocompetent children. The organism was found in the stool of 65% of symptomatic children com-

pared with 11% of asymptomatic children. Treatment does not seem to be indicated in the immunocompetent host.⁶⁰

Approach to Prevention of Infection in Day-Care Centers

The roles of the community health department, physician, day-care provider, and parents need to be defined. There should be promulgation of uniform and acceptable local and state regulations. The physician must always notify the DCC and local health department when a significant pathogen is identified.⁶¹ Day-care providers must establish and enforce health policies. It is the parent's responsibility to understand the rules of the DCC, to learn about possible disease transmission, and to support measures necessary to decrease the spread of disease.

Practical infection control measures^{62, 63} include (1) education of DCC staff especially with regard to the importance of hand-washing, (2) supervision by the DCC staff on the use of toilets and hand-washing by children, (3) exclusion from DCC of children with suspected or proven infectious disease, (4) adherence to recommended student to teacher ratios, (5) ready availability of a health coordinator and written health guidelines, and (6) exclusion of new children from a DCC during an outbreak.

References

1. Phillips SF: Diarrhea: A broad perspective. *Viewpoints Dig Dis* 1979; 7:5.
2. Fordtran JS, Ingelfinger FJ: Absorption of water, electrolytes and sugars from the human gut, in Code CF (ed): *Handbook of Physiology: Alimentary Canal*. 1968, Washington, DC, American Physiologic Society, vol 3, p 1457.
3. Debongnie JC, Phillips SF: Capacity of the colon to absorb fluid. *Gastroenterology* 1978; 74:698.
4. Cohlan SQ: Chronic nonspecific diarrhea in infants and children treated with diiodohydroxyquinolone. *Pediatrics* 1956; 18:424.
5. Davidson M, Wasserman R: The irritable colon of childhood (chronic nonspecific diarrhea syndrome). *J Pediatr* 1966; 69:1027.
6. Fenton TR, Harries JT, Milla PJ: Disordered small intestinal motility: A rational basis for toddlers' diarrhea. *Gut* 1963; 24:897.
7. Greene HL, Ghishan FK: Excessive fluid intake as a cause of chronic diarrhea in young children. *J Pediatr* 1983; 102:836.
8. Cohen SA, Hendricks KM, Mathis RK, et al: Chronic nonspecific diarrhea: Dietary relationships. *Pediatrics* 1969; 64:402.
9. Boyne LJ, Kerzner B, McClung HJ: Chronic nonspecific diarrhea: The value of a preliminary period to assess diet therapy. *Pediatrics* 1985; 76:557.
10. Lifshitz F, Ceollo-Ramirez P, Contreras-Gutierrez ML: The response of infants to carbohydrate oral loads after recovery from diarrhea. *J Pediatr* 1979; 79:612.
11. Ostrander CR, Cohen RS, Hopper AO, et al: Breath hydrogen analysis: A review of the methodologies and clinical applications. *J Pediatr Gastroenterol Nutr* 1983; 2:525.

12. Townley RRW: Disaccharidase deficiency in infancy and childhood. *Pediatrics* 1966; 38:1966.
13. Gudmand-Hoyer E: Sucrose malabsorption in children: A report of thirty-one Greenlanders. *J Pediatr Gastroenterol Nutr* 1985; 4:873.
14. Lebenthal E, Heitlinger L, Lee PC, et al: Corn syrup sugars: In vitro and in vivo digestibility and clinical tolerance in acute diarrhea of infancy. *J Pediatr* 1983; 103:29.
15. Fisher SE, Leone G, Kelly RH: Chronic protracted diarrhea: Intolerance to dietary glucose polymers. *Pediatrics* 1981; 67:271.
16. Anderson IH, Levine AS, Levitt MD: Incomplete absorption of the carbohydrate in all-purpose wheat flour. *N Engl J Med* 1981; 304:891.
17. Farley TA: Severe hyponatremic dehydration after use of an activated charcoal-sorbitol suspension. *J Pediatr* 1986; 109:719.
18. Hyams JS: " 'Sugar free' products": An unappreciated cause of gastrointestinal complaints. *Gastroenterology*. 1983; 84:30.
19. Ford RPK, Menzies IS, Phillips AD, et al: Intestinal sugar permeability: Relationship to diarrheal disease and small bowel morphology. *J Pediatr Gastroenterol Nutr* 1985; 4:568.
20. Iyngkaran N, Abdin Z, Davis K, et al: Acquired carbohydrate intolerance and cow milk protein-sensitive enteropathy in young infants. *J Pediatr* 1979; 95:373.
21. Code CF: The interdigestive housekeeper of the gastrointestinal tract. *Perspect Biol Med* 1979; 2:S49.
22. Wingate DL, Pearce EA, Hutton M, et al: Quantitative comparison of the effects of cholecystokinin, secretin, and pentagastrin on gastrointestinal myoelectric activity in the conscious fasted dog. *Gut* 1978; 19:593.
23. Coello-Ramirez P, Lifshitz F: Enteric microflora and carbohydrate intolerance in infants with diarrhea. *Pediatrics* 1972; 49:233.
24. Challacombe DN, Richardson JM, Rowe B, et al: Bacterial microflora of the upper gastrointestinal tract in infants with protracted diarrhea. *Arch Dis Child* 1974; 49:270.
25. Hill ID, Mann MD, Moore L, et al: Duodenal microflora in infants with acute and persistent diarrhea. *Arch Dis Child* 1983; 58:330.
26. Househam KC, Mann MD, Mitchell J, et al: Duodenal microflora in infants with acute diarrhea disease. *J Pediatr Gastroenterol Nutr* 1986; 5:721.
27. Fagundes-Neto U, Tocciano H, Dujorney F: Stool bacterial aerobic overgrowth in the small intestine of children with acute diarrhea. *Acta Pediatr Scand* 1976; 65:609.
28. Davidson GP, Robb TA, Kirubakaran CP: Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain: Diagnosis by breath hydrogen test. *Pediatrics* 1984; 74:229.
29. Poley JR: Chronic nonspecific diarrhea in children: Investigation of the surface morphology of small bowel mucosa utilizing the scanning electron microscope. *J Pediatr Gastroenterol Nutr* 1983; 2:71.
30. Lifshitz F: Diarrhea, in Paige D (ed): *Manual of Clinical Nutrition*. Margate, NJ, Nutrition Publications, Inc, 1983, vol 1, p. 1.
31. Heiner DC, Sears JW, Koniker WT: Multiple precipitins to cow's milk in chronic respiratory disease. *Am J Dis Child* 1962; 103:634.
32. Savilahti E, Simell O: Chronic non-specific diarrhea. *Arch Dis Child* 1985; 60:452.

33. Bahna SL, Heiner DC: Cow's milk allergy. *Adv Pediatr* 1978; 25:1.
34. Goldman SA, Anderson DW, Sellers WA, et al: Milk allergy: I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963; 32:425.
35. Lifshitz F, Carrere E: Food sensitivity and intolerance leading to diarrhea. *Clin Nutr* 1984; 3:5.
36. Powell GK: Milk and soy-induced enterocolitis of infancy. *J Pediatr* 1978; 93:553.
37. Jenkins HR, Pincott JR, Southill et al: Food allergy: The major cause of infantile colitis. *Arch Dis Child* 1984; 59:326.
38. Salazar de Sousa J, Silva A, Pereira MV, et al: Cow's milk protein-sensitive enteropathy: Number and timing of biopsies for diagnosis. *J Pediatr Gastroenterol Nutr* 1986; 5:207.
39. Kosnai I, Kuitunen P, Savilahti E, et al: Mast cells and eosinophils in the jejunal mucosa of patients with intestinal cow's milk allergy and celiac disease of childhood. *J Pediatr Gastroenterol Nutr* 1984; 3:368.
40. Maluenda C, Phillips AD, Briddon A, et al: Quantitative analysis of small intestinal mucosa in cow's milk sensitive enteropathy. *J Pediatr Gastroenterol Nutr* 1984; 3:349.
41. Wraith DG, Merett J, Roth A, et al: Recognition of food-allergic patients and their allergens by the RAST technique for clinical investigation. *Clin Allergy* 1979; 9:25.
42. Goldman AS, Sellers WA, Halpern SR, et al: Milk allergy: II. Skin testing of allergic and normal children with purified milk proteins. *Pediatrics* 1963; 32:572.
43. Ashkenazi A, Levin S, Idar D, et al: In vitro cell-mediated immunologic assay for cow's milk allergy. *Pediatrics* 1980; 32:572.
44. Halpern SR, Seller WA, Johnson RB, et al: Development of childhood allergy in infants fed breast, soy, or cow's milk. *J Allergy Clin Immunol* 1973; 51:139.
45. Whittington PF, Gibson R: Soy protein intolerance: Four patients with concomitant cow's milk intolerance. *Pediatrics* 1977; 59:730.
46. Gerrard JA: Oral cromoglycate: Its value in the treatment of adverse reactions to foods. *Ann Allergy* 1979; 42:135.
47. Lloyd-Still JD: Chronic diarrhea of childhood and the misuse of elimination diets. *J Pediatr* 1979; 95:10.
48. MacLean WC, Romana GL, Massa E, et al: Nutritional management of chronic diarrhea and malnutrition: Primary reliance on oral feeding. *J Pediatr* 1980; 97:316.
49. Schwarz RP, Ulshen MH: Pseudomembranous colitis presenting as mild, chronic diarrhea in childhood. *J Pediatr Gastroenterol Nutr* 1983; 2:570.
50. Fleisher D, Ament ME: Diarrhea, red diapers, and child abuse. *Clin Pediatr* 1977; 17:820.
51. Doyle AR: Incidence of illness in early group and family day care. *Pediatrics* 1976; 58:607.
52. Shuman SH: Day-care associated infection: More than meets the eye. *JAMA* 1983; 249:76.
53. Hadler SC: Enteric infections in day-care centers. *Ross Roundtable*, July 1985, p 64.
54. Pickering LK, Evans DG, DuPont HL, et al: Diarrhea caused by *Shigella*, rotavirus, and *Giardia* in day-care centers: Prospective study. *J Pediatr* 1981; 99:51.

55. Keystone JS, Yang H, Grisdale D, et al: Intestinal parasites in metropolitan Toronto day-care centres. *Can Med Assoc J* 1984; 131:733.
56. Bartlett AV, Moore M, Gary GW, et al: Diarrheal illness among infants and toddlers in day care centers: I. Epidemiology and pathogens. *J Pediatr* 1985; 107:495.
57. Bartlett AV, Moore M, Gary GW, et al: Diarrhea illness among infants and toddlers in day care centers: II. Comparison with day care homes and households. *J Pediatr* 1985; 107:503.
58. Lieb S, Gunn RA: Salmonellosis in a day-care center. *J Pediatr* 1982; 100:1004.
59. Paulozzi LJ, Johnson KE, Kamahele LM, et al: Diarrhea associated with adherent enteropathogenic *Escherichia coli* in an infant and toddler center, Seattle, Washington. *Pediatrics* 1986; 77:296.
60. Alpert G, Bell M, Kirkpatrick CE, et al: Outbreak of cryptosporidiosis in a day-care center. *Pediatrics* 1986; 77:152.
61. Committee on Early Childhood, Adoption, and Dependent Care: The pediatrician's role in promoting the health of a patient in day care. *Pediatrics* 1984; 74:157.
62. Haskins R, Kotch J: Day care and illness: Evidence, costs, and public policy. *Pediatrics* 1986; 77(suppl):951.
63. Hadler SC: Public health considerations of infectious diseases in child day care center. *J Pediatr* 1984; 105:683.

The Approach to Fluid and Electrolyte Therapy in Pediatrics

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The therapeutic objective in the treatment of fluid and electrolyte imbalances in infants and children is to restore normal physiologic homeostasis. To achieve this goal, recognition of the process of growth and development with regards to the changes in body composition, metabolism, and regulatory mechanisms is required. This review provides a practical approach to the clinical management of infants after the newborn period and children with disorders of fluid and electrolyte homeostasis. First, the normal requirements of water and electrolytes for maintenance of body composition during development are presented to serve as a guideline for the formulation of parenteral therapy. The subsequent sections address the pathophysiology underlying specific disorders of water and electrolyte homeostasis. The clinical and laboratory assessment of each disorder is emphasized and the appropriate therapeutic regimen is outlined.

Normal Maintenance Requirements

Maintenance therapy is the replacement of water and electrolytes normally lost through respiration, evaporation from the skin, urinary excretion, and the gastrointestinal tract.^{1, 2} The goal of this therapy is to provide these requirements, as well as substrate for nutrition, in a form that places minimal stress on renal function and prevents significant endogenous protein catabolism and ketosis. Treatment is aimed at the pediatric patient who

may have no history of abnormal losses of water and electrolytes, termed deficit losses, but who is temporarily prevented from ingesting a normal oral intake. For example, parenteral maintenance therapy is indicated for the preoperative or postoperative patient. Alternatively, maintenance therapy is part of the total parenteral fluid treatment of a patient with sustained losses of body water and electrolytes during an episode of severe gastroenteritis.

While maintenance fluid and electrolyte requirements of infants and children are based on metabolic activity,³ deficit therapy is more closely related to body weight⁴ and is used to estimate the replacement of losses. It accounts for body deficits of water, sodium, potassium, chloride, base, and nutrition.

In estimating adequate fluid and electrolyte therapy, it is important to consider the immaturity of renal function in the young infant. The postnatal maturation of renal function is characterized by increases in the rates of renal blood flow, glomerular filtration, and tubular function.⁵ The glomerular filtration rate per 1.73 sq m remains low during the first several years of life, but adequate to excrete the solutes found in breast milk and formulas. Infants have a limited ability to excrete a sodium or water load, or maximally concentrate the urine. Administration of excess hypotonic fluids result in a fluid overloaded, hyposmolar state. Alternatively, inability to maximally concentrate the urine in the presence of abnormal losses of water through evaporation, the gastrointestinal tract, or excessive renal solute loads predisposes the infant to hyperosmotic disorders.

Concomitant with the maturation of the renal regulatory systems governing water and electrolyte homeostasis are developmental changes in the distribution and composition of the body compartments. Combined with the increased metabolic requirements of water, electrolytes, acid, base, and calories, the infant and small child are vulnerable to disorders of fluid and electrolyte homeostasis when the normal balance is compromised.⁶

Underlying the principles governing water and electrolyte homeostasis are the composition and metabolic turnover of the major body compartments. The body mass is comprised of a fluid phase, or total body water (TBW) that is distributed as extracellular fluid (ECF) and intracellular fluid (ICF), and a solid phase consisting of tissue cells, bone, and fat. This distribution in a fat-free infant is illustrated in Figure 1.⁷ During development, significant changes occur in the TBW and body compartments as fat, skeletal and muscle content contribute more to the total body mass (Table 1).^{8, 9}

The composition of the fluid and solid phases of the body is illustrated in Figure 2. It is evident that despite differences in the ionic composition of the various body fluid compartments, the osmolality within these compartments is equal. Mechanisms responsible for this osmolar equilibration involve selective membrane permeabilities of the different compartments and active transport processes. These factors determine the movement of water throughout the compartments during states of altered body fluid osmolality associated with disorders of fluid and electrolytes.

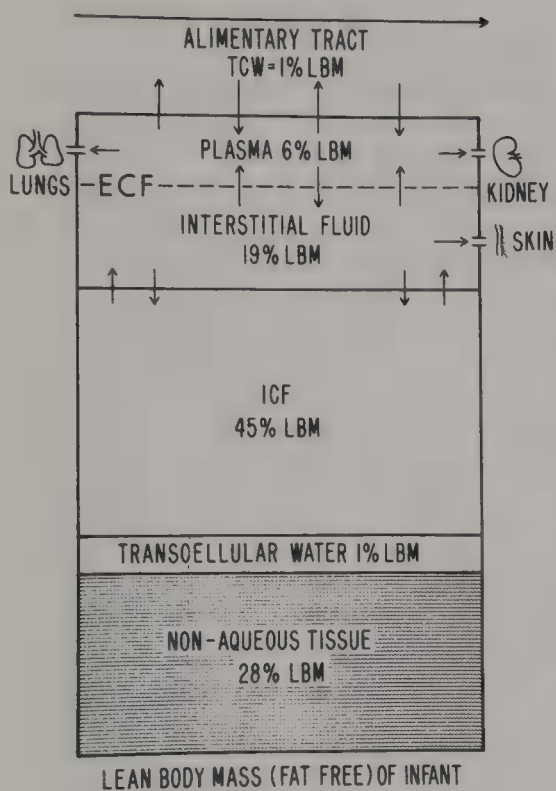


FIG 1. Body fluids in the infant as a proportion of the lean body mass (LBM), showing the rapid movement of water molecules (arrows) among compartments. Transcellular water (TCW) is comprised of that in alimentary and urinary tracts as well as inaccessible cartilage and bone water. Cerebrospinal fluid is included in interstitial fluid. ECF = extracellular fluid; ICF = intracellular fluids. (From Finberg L, Kravath RE, Fleischman AR (eds): *Water and Electrolytes in Pediatrics, Physiology, Pathophysiology and Treatment*. Philadelphia, WB Saunders Co, 1982. Used by permission.)

The process of growth requires a higher metabolic rate per unit body weight and is most obvious during the first 2 years of life.⁴ Water requirements are related to energy metabolism and are due to loss of water through heat production. Body mass and surface area are important factors determining heat production and heat loss,⁷ and a relationship can be made between body weight and caloric expenditure.¹ The infant's surface

TABLE 1.
Changes in Total Body Water (TBW) and Body Compartments During Development

Age	TBW (% Body Weight)	Extracellular fluid (% Body Weight)	Intracellular Fluid (% Body Weight)
Premature	75–80		
Newborn	70–75	50	35
1 year	65	25	40–45
Adolescence			
Males	60	20	40–45
Females	55	18	40

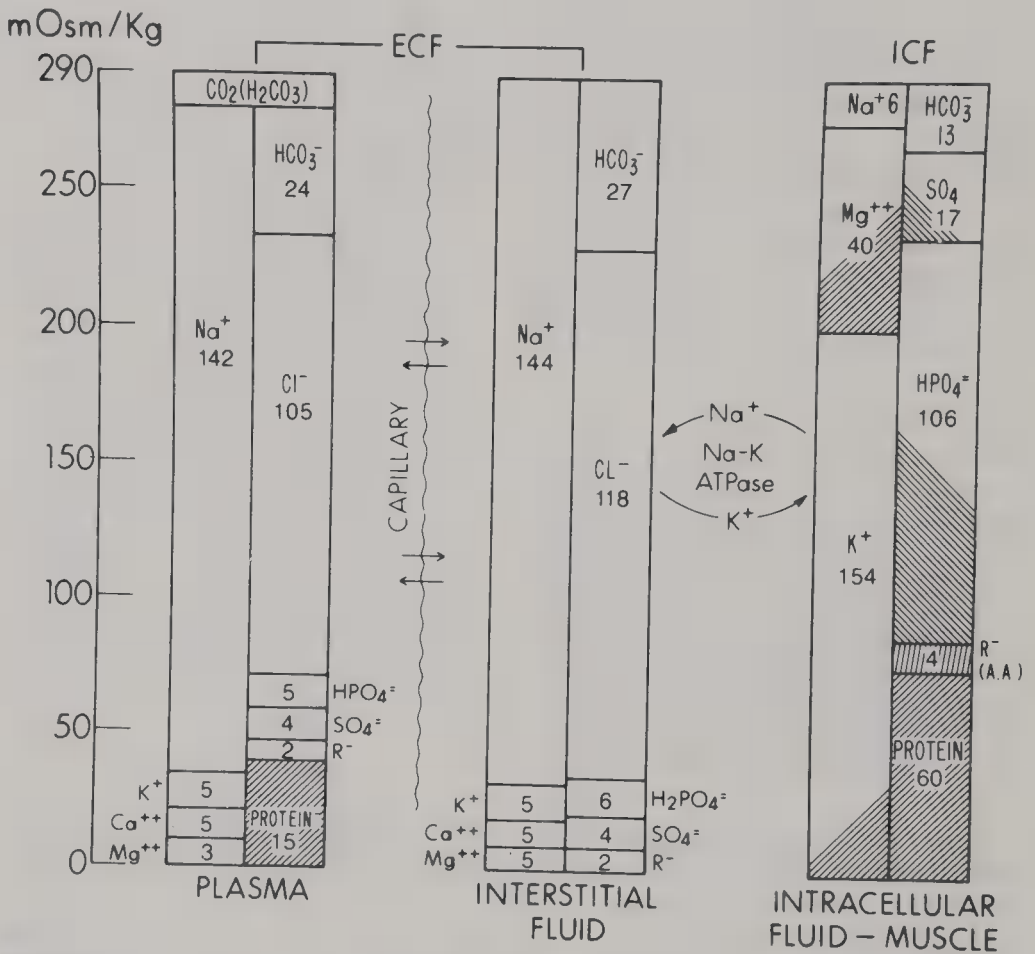


FIG 2.

Ionic profiles of body fluids: approximate representation of cations and anions of the three principal body fluid compartments. All are electrically neutral and have same osmolality despite differences in total charges. Shaded areas represent large molecules or bound ions whose osmolal contribution (mOsm/kg) is much smaller than their electrical charge but which are of importance to the distribution of ions because of their impermeability. ECF = extracellular fluid; ICF = intracellular fluids. (From Finberg L, Kravath RE, Fleischman AR (eds): *Water and Electrolytes in Pediatrics, Physiology, Pathophysiology and Treatment*. Philadelphia, WB Saunders Co, 1982. Used by permission.)

area to mass ratio is fivefold greater than that of the adult, and it must produce more heat per unit mass to remain in thermal balance.⁶

These considerations provide the empiric foundation for the major fluid and electrolyte regimens used in the calculation of maintenance therapy. They differ since they are based on either the caloric expenditure, body surface area, or body weight. Estimates based on surface area are good regardless of body size, and in children with normal height for weight relationships, weight alone can be used to estimate the surface area.⁹ How-

ever, the relationship between surface area and metabolism is variable in premature and newborn infants. Methods based on the body weight require a reduction in dose with increasing age and size.

It is convenient to relate maintenance metabolic water losses in milliliters to energy expended in calories,³ with 1 ml of water being lost for each calorie metabolized. Calories expended, in turn, can be related to the body weight at any particular age. These metabolic water losses are comprised of water lost from the body as insensible, urinary, and that found in stool. The relationship also considers factors that may alter the metabolic rate.

Evaporative water losses through the skin and respiration comprise insensible losses of electrolyte-free water. Under the normal baseline state, these values approximate 30 and 15 ml/100 calories expended, respectively. Skin losses increase with caloric expenditure as in muscular activity or shivering, as does the electrolyte content of sweat. For each degree Centigrade elevation in body temperature above normal, there is a 10% increase in caloric expenditure.¹⁰ A high environmental temperature increases water loss while an elevated ambient humidity decreases water dissipation from the skin and lungs. Lung losses vary with the respiratory rate. The water lost as obligatory urine output is a consequence of the renal excretion of waste products of protein metabolism as urea, and other electrolyte salts. The final urine osmolality reflects the renal solute load of osmotically active particles as well as the kidney's reabsorption or excretion of salt and water. These losses approximate 50 ml/100 calories expended provided the urine osmolality is isoosmotic and equal to that in the ECF. Fluid therapy based on the aforementioned requirement will place minimal osmotic work on the kidneys. An additional 5 ml/100 calories is obligated in stool water. Thus, a total of 100 ml/100 calories expended accounts for the maintenance water losses under baseline conditions.

When calculating normal maintenance water requirements, allowance should be made for endogenously generated water, especially in the presence of renal failure or congestive heart failure. One source of endogenous body water is oxidation of carbohydrate or fat, which yields carbon dioxide and water. The water of oxidation averages 12 to 17 ml/100 calories metabolized. Another source, called preformed water, approximates 3 ml/100 calories and is derived from tissue catabolism during disease states. If sweating is minimal, then sweat and stool losses are roughly balanced by these endogenous sources.

Maintenance requirements for electrolytes are part of the parenteral therapy needed to maintain body fluid homeostasis. The electrolyte intake of normal infants can be expressed in terms of caloric intake or expenditure. Human milk provides 1.0 to 1.5 mEq/100 calories of sodium and potassium, while cow's milk contains two to three times this quantity.¹¹ A generous estimation for electrolyte maintenance therapy is 2.5 mEq of sodium and potassium per 100 calories expended given as the chloride salts.¹² Exceptions include unusual ongoing losses of electrolytes via sweating, vomiting and diarrhea, surgical drainage tubes, burns, diuretic therapy,

TABLE 2.
Maintenance Water and Electrolyte Requirements Based on
Caloric Expenditure

Body Weight (kg)	Calories Expended (calories/kg/day*)	Water Required (ml/100 calories/day)	Electrolytes Required (mEq/100 calories/day)
3-10	100	Insensible Skin = 30 Lungs = 15	Na = 2.5-3.0
10-20	1,000 calories + 50 per each kg > 10	Renal = 0 Stool = 5	K = 2.0-2.5 Cl = 2.0
>20	1,500 calories + 20 per each kg > 20		

Total = 100 ml/100 cal

*Assuming that normal maintenance water requirement is 100 ml/100 calories, calculate maintenance fluid directly in ml/kg.

and renal electrolyte losing disorders. Determination of the electrolyte composition of abnormal fluid losses is required to arrive at appropriate parenteral therapy.

One can estimate the daily maintenance requirements for water, electrolytes, and nutrition by calculating the caloric expenditure. This is illustrated in Table 2 assuming basal metabolic expenditure is increased only slightly during bed rest. Metabolic activity, and therefore caloric requirements, change with stress so that these factors (e.g., fever, surgery, exercise, burns, diabetes insipidus) as well as factors that increase gastrointestinal or sweat losses need to be taken into account and water requirements adjusted accordingly. Table 3 shows maintenance requirements based on body surface area.

The nutritional component of maintenance therapy provides substrate for metabolism. Optimal nutritional therapy provides an equal number of calories for those expended. However, for short-term maintenance therapy in a previously well-nourished infant or child, a combination of parenteral nutrition and the patient's own fat stores are adequate to prevent significant ketosis and tissue catabolism. This is accomplished by administering sufficient glucose equal to approximately 20% of the total caloric expenditure, or 5 gm of glucose per 100 calories expended (60 gm sq m/day). In the event of a more chronic condition, especially in the undernourished child, nutritional therapy should provide the required calories via parenteral and/or enteral administration of carbohydrate, fat, amino acids, minerals, and vitamins.

TABLE 3.
Maintenance Water and Electrolyte Requirements Based on Square Meter of
Surface Area

Body Weight (kg)	Sq m	Water Required (ml/sq m/day)	Electrolytes Required (mEq/sq m/day)
0-5	0.05 × BW + 0.05	insensible = 600-900	Na = 30-50
6-10	0.04 × BW + 0.10	renal = 400-750	
11-20	0.03 × BW + 0.20	stool = 50	K = 30-40
21-30	0.02 × BW + 0.40		Cl = 30
		Total = 1,500 ml/sq m/day	

An appropriate solution for average maintenance therapy contains 25 to 30 mEq/L of Na and 20 mEq/L of K as the chloride salts in 5% dextrose and water. This solution can be modified to meet unusual requirements for water and electrolytes as needed.

Dehydration

Dehydration can be simply defined as negative body fluid or water balance. The assessment of the degree of diminution of body fluids is made by both subjective and objective criteria. The clinician formulates a therapeutic plan based on the degree of dehydration, nature of resultant body fluid osmolality (isotonic, hypotonic, hypertonic), and size of the child.

Negative water balance occurs by a decreased intake or increased loss of fluid.

Dehydration due to inadequate intake is primarily caused by unreplaced normal insensible water loss. Examples include anorexia; dysphagia; comatose state; and infants or patients who are debilitated, restrained, or with impaired thirst.

Increased loss occurs with excessive insensible water loss (e.g., burns, cystic fibrosis, sweating, fever, increased ambient temperature, and hyperventilation) and urinary or gastrointestinal diseases. Infants and children with tracheostomies have an increased pulmonary water loss due to a reduction in the dead air fraction of the tidal volume.¹³ It has been estimated that patients with moderate sweating lose between 400 and 2,000 ml of water per sq m per day with a sodium concentration of only 10 to 30 mEq/L.^{13, 14} In contrast, sodium losses in children with cystic fibrosis may vary between 50 and 130 mEq/liter.¹⁵

Increased renal water loss occurs secondary to osmotic diuresis, central diabetes insipidus, impaired tubular response to antidiuretic hormone, and sodium-wasting conditions. The osmotic diuretic, mannitol, may increase the urine output to 10 ml/min with sodium and potassium concentrations of 90 and 15 mEq/L, respectively.¹⁶ The states associated with excessive water loss from renal tubular dysfunction include chronic pyelonephritis, recovery or diuretic phase of intrinsic acute renal failure, diuretic period that follows relief of urinary tract obstruction, hypokalemia, hypercalcemia, sickle cell disease, or congenital renal disease.

Gastrointestinal water loss from diarrheal disease is the most common cause of dehydration in infants and children. Ileostomy losses and vomiting are also frequent in the pediatric population. The pathophysiology and treatment of dehydration secondary to diarrhea is the main focus of this section.

Classically, dehydration has been divided into three types based on the serum sodium concentration. Isonatremic dehydration is present when the serum sodium concentration is normal (130 to 150 mEq/L); hyponatremic

dehydration when the serum sodium concentration is less than 130 mEq/L; and hypernatremic dehydration when the serum sodium exceeds 150 mEq/L.

In general, fluid losses in dehydration are hypotonic compared to the ECF. However, all three types of dehydration can be generated from the same type of fluid loss. In isonatremic dehydration, there is an equal loss of sodium and potassium. The potassium deficit is the result of losses from the ICF, whereas the sodium deficit is from the ECF. However, a portion of the sodium loss is the result of movement to the ICF to compensate for the potassium loss to maintain electroneutrality. The resultant body fluid composition is isotonic with no significant change in extracellular fluid osmolality. Since there is no osmolar gradient between the ECF and ICF, the volume deficit is entirely from the ECF.

In hypotonic dehydration, there is a proportionally greater loss of sodium and potassium compared to fluid loss. This initial fluid loss reduces the volume and osmolality of the ECF below that of the ICF. Because of this osmolar gradient, fluid moves from the ECF to the ICF. This readjustment has two major effects: (1) both the ECF and ICF become hypotonic; and (2) ECF volume is reduced at the expense of ICF expansion. This ECF reduction is larger than would be expected from the disease-associated losses. This severe contraction in ECF volume increases the likelihood of underperfusion to the vital organs.

In hypertonic dehydration, there is a proportionally greater loss of fluid than electrolytes. Due to the fluid losses, the ECF is initially contracted and hypertonic. To maintain osmolar equilibrium, fluid moves from the ICF to the ECF. The end result is a hypertonic ECF and ICF. This relative expansion of the ECF volume may lead to an underestimation of the degree of dehydration. Based on this consideration, the clinical assessment and management need to be modified to account for variations in the serum sodium concentration.

Clinical Assessment

It is essential to obtain a complete history to document the voiding history (wet diapers, etc.) and the type, volume, and frequency of the abnormal losses. It is equally important to document the type, volume, and frequency of oral replacement fluids consumed prior to the hospital visit. An estimate of the degree of dehydration is best made by determining the weight loss associated with the illness. Knowledge of the pre-illness weight and the patient's admission weight permits an estimate of the fluid losses.

$$\text{Degree of Dehydration (\%)} = \frac{\text{Pre-illness Weight} - \text{Admission Weight}}{\text{Pre-illness Weight}} \times 100\%$$

All the other symptoms and signs of dehydration are only reasonable

TABLE 4.
Clinical Assessment of Severity of Dehydration*

Sign and Symptoms	Mild Dehydration	Moderate Dehydration	Severe Dehydration
General appearance and condition			
Infants and young children	Thirsty, alert, restless	Thirsty, restless, or lethargic but irritable to touch or drowsy	Drowsy, limp, cold, sweaty, cyanotic extremities, may be comatose
Older children and adults	Thirsty, alert, restless	Thirsty, alert, postural hypotension	Usually conscious, apprehensive, cold, sweaty, cyanotic extremities, wrinkled skin of fingers and toes, muscle cramps
Radial pulse	Normal rate and strength	Rapid and weak	Rapid, feeble, sometimes not palpable

Respiration	Normal	Deep, may be rapid	Deep and rapid
Anterior fontanelle	Normal	Sunken	Very sunken
Systolic blood pressure	Normal	Normal or low	Less than 90 mm Hg, may be unrecordable
Skin elasticity	Pinch retracts immediately	Pinch retracts slowly	Pinch retracts very slowly (2 seconds)
Eyes	Normal	Sunken	Grossly sunken
Tears	Present	Absent	Absent
Mucous membranes	Moist	Dry	Very Dry
Urine flow	Normal	Reduced amount and dark	None passed for several hours, empty bladder
% body weight loss	5%	10%	15%
Estimated fluid deficit	50 ml/kg	100 ml/kg	150 ml/kg

*From Robson A: Parental fluid therapy, in Behrman RE, Vaughan VC (eds): *Nelson's Textbook of Pediatrics*, ed 13. Philadelphia, WB Saunders Co, 1987, p 196. Used by permission.

assessments largely influenced by interobserver variation (Table 4), but are all that may be available in the absence of reliable measurements of the pre-illness weight. In *mild dehydration*, the blood pressure and pulse are normal. Other symptoms and signs of dehydration are usually absent. In *moderate dehydration*, the pulse rate is increased but the blood pressure is usually normal, although postural hypotension may be present. In children under 18 months of age, the anterior fontanelle may be sunken. Other key findings are the lack of tears, sunken eyes, dry mucous membranes, and decreased abdominal skin elasticity. Skin elasticity is detected by retraction of the abdominal skin when pinched with the thumb and index finger. In contrast, turgor is related to normal capillary filling after pinching the skin. Decreased turgor is associated with significant circulatory or intravascular compromise.

Patients with *severe dehydration* have circulatory collapse with significant central nervous system compromise ranging from lethargy to coma. The extremities are cool and may be cyanotic. The pulse is rapid and thready, and the blood pressure is low for age (systolic usually under 80 mmHg). The fontanelle is severely depressed. Tears are absent and the eyes are sunken with prominent dark rings. Severe dehydration mandates aggressive intravenous administration of a plasma expander to prevent significant low perfusion injury to the brain, liver, and kidneys.

Certain key physical findings permit the differentiation of the type of dehydration (Table 5). With equivalent degree of dehydration, the child with hyponatremic dehydration has a proportionately lower intravascular/extracellular fluid volume than in isonatremia or hypernatremia. This will be associated with earlier circulatory compromise compared with the other types of dehydration. In hypernatremic dehydration, the child may appear lethargic while lying on the examining table, but becomes hyperirritable when aroused. In addition, the skin feels velvety or doughy. Many patients with moderate to severe dehydration may have nuchal rigidity suggesting meningeal infection.

Therapy

The treatment plan for dehydration is formulated at the time of clinical assessment. Phase I or emergency therapy is directed to restore intravascular volume (Fig 3). The choice of fluid consists of isotonic (0.9%) saline, lactated Ringer's, 5% albumin (Plasmanate), 5% dextrose with 0.45% isotonic saline, or whole blood. Isotonic saline and Ringer's are readily available in the emergency room. The fluid is given over 1 hour at a rate of 20 ml/kg. If the patient is in shock, the fluid is given as rapidly as possible until blood pressure is normalized and the pulse becomes stronger. If the patient does not respond appropriately to 20 ml/kg, then an additional push of 20 ml/kg should be administered to restore the integrity of the circulation. Patients with mild dehydration do not necessarily require rapid

TABLE 5.
Effects of Type of Dehydration on Physical Signs*

	Isonatremic Dehydration	Hyponatremic Dehydration	Hypernatremic Dehydration
ECF volume	Markedly decreased		
ICF volume	Maintained	Severely decreased	Decreased
Physical signs		Increased	Decreased
Skin			
Color†	Gray	Gray	Gray
Temperature	Cold	Cold	Cold or hot
Turgor‡	Poor	Very poor	Fair
Feel	Dry	Clammy	Thickened, doughy
Mucous membrane	Dry	Slightly moist	Parched§
Eyeball	Sunken and soft	Sunken and soft	Sunken
Fontanelle	Sunken	Sunken	Sunken
Psyche	Lethargic	Coma	Hyperirritable
Pulse†	Rapid	Rapid	Moderately rapid
Blood pressure†	Low	Very low	Moderately low

*From Robson A: Parental fluid therapy, in Behrman RE, Vaughn VC (eds): *Nelson's Textbook of Pediatrics*, ed 13. Philadelphia, WB Saunders Co, 1987, p. 196. Used by permission.

†Signs of shock rather than of dehydration itself.

‡Reflects magnitude of fluid loss from ECF.

§Tongue often has shriveled appearance owing to loss of cellular fluid.

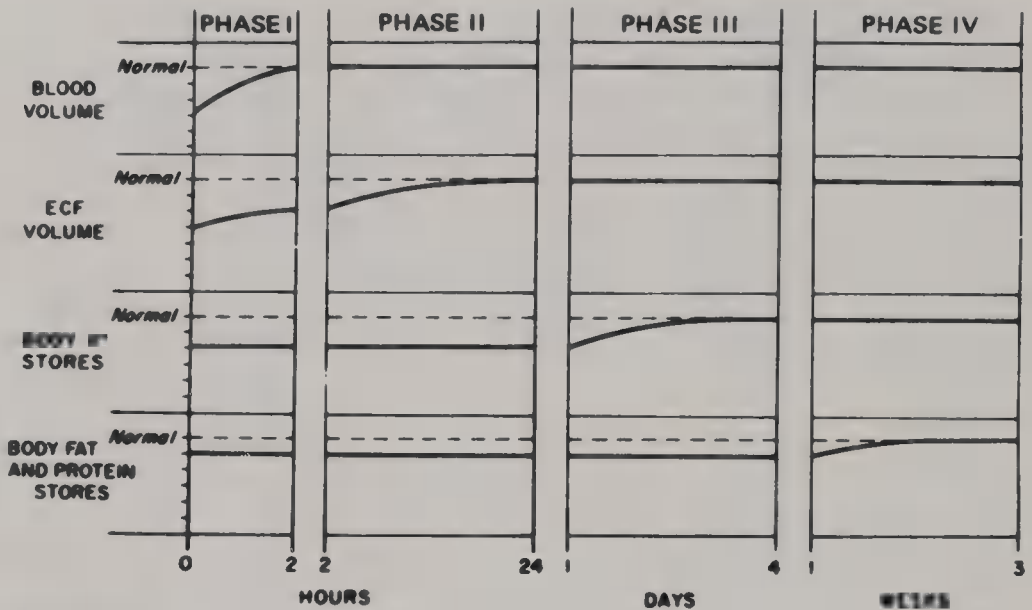


FIG 3.

The chronological sequence of the four phases of fluid therapy in a patient with dehydration. ECF = extracellular fluid. (From Winters RW (ed): *Principles of Pediatric Fluid Therapy*, ed 2. Boston, Little, Brown & Co, 1982. Used by permission.)

volume expansion. However, the decision to omit phase I therapy has to be individualized.

Phase II (repletion or restoration therapy) occurs over the first 24 hours following admission to the hospital. In most cases, this phase begins when the patient reaches the inpatient floor. The volume of fluid administered consists of the maintenance requirements plus replacement of estimated deficits and ongoing losses. Deficit is defined as negative balance of water and electrolytes that occur secondary to a disease condition. The type of fluid is based on the type of dehydration. However, 5% dextrose with 0.45% isotonic saline is a satisfactory choice for most situations (see later). During this phase of restoring extracellular fluid volume, it is important to monitor serum chemistries, body weight, and urine output every 12 hours to constantly reassess the adequacy of therapy.

Phase III coincides with the initiation of oral intake of fluids, carbohydrates, protein, and fats after the first 24 hours. The body stores of potassium, fat, and protein are replenished. However, enteral therapy may not be possible in those children with hypernatremic dehydration due to the need for slow steady rehydration and those with significant ongoing intestinal losses.

TABLE 6.
Conditions Resulting in the Abnormal Loss of Water and Electrolytes*

Fluid	Na + (mEq/L)	K + (mEq/L)	Cl - (mEq/L)	HCO ₃ - (mEq/L)
Sweat-heat exhaustion, fever	50	0-15	50	0
Gastric juice	50	5-15	110	0
Pancreatic juice	140	5	75	110
Small bowel	140	5	110	30
Ileostomy	130	10	110	30
Diarrhea	50-140	5-15	55-100	15-50

*The values represent a composite from references 7, 13, 18, and 19.

Specific Therapy for Isonatremic Dehydration

Phase I

20 to 40 ml/kg of isotonic saline, lactated Ringer's, 5% albumin, 5% dextrose with 0.45% isotonic saline

Phase II

A. Maintenance volume and electrolytes for 24 hours

B. Calculated deficit*

Volume = 8 ml/kg/% dehydration

Sodium = 10.8 mEq/kg/% dehydration

Potassium† = 0.3 mEq/kg/% dehydration

C. Ongoing losses (diarrhea, etc.) (Table 6)^{7, 13, 18, 19}

Volume of loss in ml is added to the calculated 24-hour volume and administered in a similar electrolyte composition.

The total phase II volume (maintenance + deficit + ongoing losses) is administered as follows:

1. Provide 50% over the first 8 hours.

2. Provide remaining 50% over subsequent 16 hours.

*The calculated volume deficit assumes phase I therapy has been administered. In the case where phase I fluid administration is not necessary, the calculated deficit fluid should be 10 ml/kg/% dehydration. In addition, the weight in kg is the pre-illness weight, if available. These deficit calculations were derived and modified from references 1, 7, and 17.

†The calculated potassium deficit should not be given until the patient has voided. At that point, approximately 50% of the actual deficit is given due to the danger of large intravenous doses of potassium.

Phase III

Begin to switch from IV to PO fluids and include any ongoing losses.

Example

A 5-year-old girl has dehydration secondary to diarrheal losses. Estimated pre-illness weight is 20 kg; present weight is 18 kg. Serum sodium concentration is 140 mEq/L. Clinical assessment:

$$\frac{20 \text{ kg} - 18 \text{ kg}}{20 \text{ kg}} \times 100\% = 10\% \text{ (moderate) isonatremic dehydration}$$

Phase I

Isotonic saline = 20 ml/kg; weight = 20 kg
 $20 \text{ kg} \times 20 \text{ ml/kg} = 400 \text{ ml over first hour}$

Phase II**A. Maintenance**

Volume = 100 ml/kg/day for first 10 kg:
 $100 \text{ ml/kg} \times 10 \text{ kg} = 1,000 \text{ ml}$
 50 ml/kg/day for next 10 kg:
 $50 \text{ ml/kg} \times 10 \text{ kg} = 500 \text{ ml}$

Total maintenance volume/24 hours = 1,500 ml

Sodium = $2.5 \text{ mEq/100 calories/day} \times 1,500 \text{ calories/day}$
 $= \frac{1,500}{100} \text{ calories} \times 2.5 \text{ mEq/day}$
 $= 37.5 \text{ mEq/day}$

Potassium = $2.5 \text{ mEq/100 calories} \times 1500 \text{ calories/day}$
 $= \frac{1,500}{100} \text{ calories} \times 2.5 \text{ mEq/day}$
 $= 37.5 \text{ mEq/day}$

B. Calculated deficit = $\frac{20 \text{ kg} - 18 \text{ kg}}{20 \text{ kg}} \times 100\% = 10\% \text{ deficit}$

Volume = $8 \text{ ml/kg/\%} = 8 \text{ ml} \times 20 \text{ kg} \times 10 = 1,600 \text{ ml}$

Sodium = $0.8 \text{ mEq/kg/\%} = 0.8 \text{ mEq} \times 20 \text{ kg} \times 10\%$
 $= 160 \text{ mEq}$

Potassium = $0.3 \text{ mEq/kg/\%} = 0.3 \text{ mEq} \times 20 \text{ kg} \times 10\%$
 $= 60 \text{ mEq}$

C. No ongoing losses

Total 24-hour volume requirement = Maintenance + deficit = 1,500
 $+ 1,600 = 3,100 \text{ ml} = 3.1 \text{ L}$

Total 24-hour sodium requirement = Maintenance + deficit = 37.5 +
 $160 = 197.5 \text{ mEq}$

Total 24-hour potassium requirement = Maintenance + deficit = 37.5
 $+ 60 = 97.5 \text{ mEq}$

Solution of choice = 5% dextrose with 197.5/mEq sodium chloride/3.1 L + 97.5 mEq potassium chloride/3.1 L = 5% dextrose with 64 mEq sodium chloride/L + 30 mEq potassium chloride/L

Final solution = 5% dextrose with 0.45% isotonic saline + 30 mEq potassium chloride/L

For hours 2 to 8 = 50% of 3,100 ml = 1550 ml/7 hours = 221 ml/hour

For hours 9 to 24 = 1550 ml for 16 hours = 97 ml/hour

Specific Therapy for Hyponatremic Dehydration

The plan is identical to isonatremic dehydration except that in phase II, additional sodium should be added to the calculated sodium deficit if the serum sodium concentration is less than 125 mg/dl.

Sodium required = (Ideal plasma sodium concentration – Actual plasma sodium concentration) \times Total body water in L/kg body weight = (Ideal plasma sodium concentration – Actual plasma sodium concentration) \times 0.6 L/kg body weight.

Example

An 8-month-old female has 10% dehydration secondary to a viral gastroenteritis. Present weight is 7.2 kg; estimated pre-illness weight is 8.0 kg. Serum sodium concentration is 123 mEq/L. Clinical assessment:

$$\frac{8.0 \text{ kg} - 7.2 \text{ kg}}{8.0 \text{ kg}} \times 100\% = 10\% \text{ (moderate) hyponatremic dehydration.}$$

Phase I

Isotonic saline = 20 ml/kg; weight = 8 kg

8 kg \times 20 ml/kg = 160 ml of isotonic saline over first hour

Phase II

A. Maintenance

Volume = 100 ml/kg/day = 100 ml/kg \times 8kg = 800 ml/day

Sodium = 2.5 mEq/100 calories $\times \frac{800}{100}$ calories \times 2.5 mEq/day
= 20 mEq/day

Potassium = 2.5 mEq/100 calories = $\frac{800}{100}$ calories \times 2.5 mEq/day
= 20 mEq/day

B. Deficit (10%)

Volume = 8 ml/kg/% = 8 ml/kg \times 8 kg \times 10 = 640 ml

Sodium = 0.8 mEq/kg/% = 0.8 mEq/kg \times 8 kg \times 10 = 64 mEq

Additional sodium = (Ideal plasma sodium – actual plasma sodium) \times 0.6 L/kg \times kg body weight = (130 mEq/L – 123 mEq/L) \times 0.6 L/kg \times 8 kg
= 33.5 mEq

Total sodium deficit = $64 + 33.6 = 98$ mEq deficit

Potassium = $0.3 \text{ mEq}/\%/ \text{kg} = 0.3 \text{ mEq}/\text{kg} \times 8 \text{ kg} \times 10 = 24$ mEq

C. No ongoing losses

Total 24-hour fluid requirement = Maintenance + deficit = $800 \text{ ml} + 640 \text{ ml} = 1,440 \text{ ml} = 1.44 \text{ L}$

Total 24-hour sodium requirement = Maintenance + deficit = $20 \text{ mEq} + 98 \text{ mEq} = 118 \text{ mEq}$

Total 24-hour potassium requirement = Maintenance + deficit = $20 \text{ mEq} + 24 \text{ mEq} = 44 \text{ mEq}$

Solution of choice = 5% dextrose with 118 mEq sodium chloride/1.44 L + 44 mEq potassium chloride/1.44 L = 5% dextrose with 82 mEq sodium chloride/L + 30 mEq potassium chloride/L

Final solution = 5% dextrose with 0.45% isotonic saline + 30 mEq potassium chloride/L

For hours 2 to 8 = $720 \text{ ml}/7 \text{ hours} = 103 \text{ ml}/\text{hour}$

For hours 9 to 24 = $720 \text{ ml}/16 \text{ hours} = 45 \text{ ml}/\text{hour}$

Specific Therapy for Hypernatremic Dehydration

Phase I

In mild cases of hypernatremic dehydration, this phase may be omitted. Otherwise, use the plan outlined under isonatremic dehydration. The rate of drop in the serum sodium concentration should not exceed 10 to 12 mEq/day.

Phase II

A. Maintenance volume and electrolyte requirements for 48 hours

B. Calculated deficit

Volume = $8 \text{ ml}/\text{kg}/\% \text{ dehydration}$

Sodium = $0.8 \text{ mEq}/\text{kg}/\% \text{ dehydration}$

Potassium = $0.15 \text{ mEq}/\text{kg}/\% \text{ dehydration}$

C. Ongoing losses

Total fluid volume should be administered over at least 48 hours.

Maintenance \times 2 days + deficit + ongoing losses = Fluid requirement divided by 48 hours.

Example

A 4-month-old male has a viral syndrome for 5 days. Weight is 4.5 kg; estimated pre-illness weight is 5 kg. Serum sodium concentration is 160 mEq/L. Clinical assessment: $\frac{5 \text{ kg} - 4.5 \text{ kg}}{5 \text{ kg}} = 10\%$ (moderate) hypernatremic dehydration

Phase I

None

Phase II

A. Maintenance

$$\text{Volume} = 100 \text{ ml/kg/day} = 100 \text{ ml/kg} \times 5 \text{ kg} = 500 \text{ ml/day}$$

$$\text{Sodium} = 2.5 \text{ mEq/100 calories/day} = \frac{500}{100} \text{ calories} \times 2.5 \text{ mEq/day} = 12.5 \text{ mEq/day}$$

$$\text{Potassium} = 2.5 \text{ mEq/100 calories} = \frac{500}{100} \text{ calories} \times 2.5 \text{ mEq/day} = 12.5 \text{ mEq/day}$$

B. Deficit (10%)

$$\text{Volume} = 8 \text{ ml/kg/\%} = 8 \text{ ml/kg} \times 5 \text{ kg} \times 10 = 400 \text{ ml}$$

$$\text{Sodium} = 0.8 \text{ mEq/kg/\%} = 0.8 \text{ mEq/kg} \times 5 \text{ kg} \times 10 = 40 \text{ mEq}$$

$$\text{Potassium} = 0.15 \text{ mEq/kg/\%} = 0.15 \text{ mEq/kg} \times 5 \text{ kg} \times 10 = 7.5 \text{ mEq}$$

C. No ongoing losses

$$\begin{aligned} \text{Total 48-hour volume requirement} &= \text{Maintenance/24 hours} \times 48 \text{ hours} + \text{deficit} = 500 \text{ ml/24 hours} \times 48 \text{ hours} + 400 \text{ ml} \\ &= 1,400 \text{ ml/48 hours} = 30 \text{ ml/hour (1.4 L/48 hours)} \end{aligned}$$

$$\begin{aligned} \text{Total 48-hour sodium requirement} &= \text{Maintenance/24 hours} \times 48 \text{ hours} + \text{deficit} = 12.5 \text{ mEq/24 hours} \times 48 \text{ hours} + 40 \text{ mEq} \\ &= 65 \text{ mEq} \end{aligned}$$

$$\begin{aligned} \text{Total 48-hour potassium requirement} &= \text{Maintenance/24 hours} \times 48 \text{ hours} + \text{deficit} = 12.5 \text{ mEq/24 hours} \times 48 \text{ hours} + 7.5 \text{ mEq} \\ &= 32.5 \text{ mEq} \end{aligned}$$

Solution of choice = 5% dextrose with 65 mEq sodium chloride/1.4 L + 32.5 mEq potassium chloride/1.4 L = 5% dextrose with 50 mEq sodium chloride/L + 25 mEq potassium chloride/L

Final solution = 5% dextrose with 0.33% isotonic saline + 25 mEq of potassium chloride*/L at a rate of 30 ml per hour for 48 hours. Alternatively, a solution of 5% dextrose with 0.45% isotonic saline + 25 mEq of potassium chloride/L can also be used.

Note: Hyperglycemia due to relative insulin deficiency is present in some cases. Many authors, therefore, recommend the use of a 2% to 3% dextrose solution rather than one of 5%.²⁰ Even though the association of hypocalcemia with hypernatremic dehydration is uncertain, it may be reasonable to add 1,000 mg of a 10% calcium gluconate solution to 500 ml of intravenous fluid.

Hypernatremic dehydration must be treated by a slow and uniform rate of fluid administration. The total 48-hour volume (maintenance \times 2 +

*Some authors recommend a potassium chloride concentration up to 40 mEq/L.²⁰

TABLE 7.
Summary for Dehydration

ISONATREMIC

Phase I

Emergency Therapy

Phase II

A. Maintenance volume and electrolytes

B. Deficit

Volume = 8 ml/kg body weight/% dehydration

Sodium = 0.8 mEq/kg body weight/% dehydration

Potassium = 0.3 mEq/kg body weight/% dehydration

C. Ongoing losses

Replace 50% of total calculated volume over first 8 hours.

Replace remaining 50% over subsequent 16 hours.

Phase III

Gradual switch to oral fluids

HYPONATREMIC

Phase I

Emergency Therapy

Phase II

A. Maintenance volume and electrolytes

B. Deficit

Volume = 8 ml/kg body weight/% dehydration

Sodium = 0.8 mEq/kg body weight/% dehydration

Additional Sodium = (Ideal plasma sodium - actual plasma sodium) \times 0.6 L/kg \times kg body weight

Potassium = 0.3 mEq/kg body weight/% dehydration

C. Ongoing losses

Replace 24-hour volume identical to isonatremic

Phase III

Gradual switch to oral fluids

HYPERNATREMIC

Phase I

Emergency therapy (variable)

Phase II

A. Maintenance volume and electrolytes for 48 hours

B. Deficit

Volume = 8 ml/kg body weight/% dehydration

Sodium = 0.8 mEq/kg body weight/% dehydration

Potassium = 0.15 mEq/kg body weight/% dehydration

C. Ongoing losses

Replace 48-hour volume at a rate of 1/48 per hour

Phase III

Gradual switch to oral fluids after 48 hours

deficit) is given in aliquots of 1/48 per hour. During the development of the hypernatremic state, idiogenic osmols (taurine, aspartate, glutamate) are formed in brain cells in order to maintain osmotic equilibrium between the extracellular and intracellular compartment.²¹ If fluids with very low sodium concentrations are administered too rapidly, osmotic adjustments cause an increase in the volume of brain cells. This cerebral volume enlargement is responsible for the neurologic manifestations observed in inappropriately treated infants with hypernatremic dehydration (Table 7).

Oral Rehydration in Developed Countries

In patients with diarrheal dehydration who are not in shock and are able to consume oral fluids, oral hydration therapy may be appropriate. Oral electrolyte solutions containing 2.0% to 2.5% glucose are given to infants and children with mild to moderate (5.0% to 10%) dehydration. The recommendations for maintenance and rehydrating solutions from the American Academy of Pediatrics Committee on Nutrition are shown in Table 8.²² The sodium concentration in the rehydrating solution is 90 mEq/L. In contrast, the concentration of sodium in the maintenance solution is 40 to 60 mEq/L. This reduction is to prevent edema formation with high fluid intakes of high sodium concentration and to reduce the risk of hypernatremia in children with a low fluid intake.²³

It is recommended that the use of an oral rehydrating solution for the treatment of mild to moderate diarrheal dehydration should follow these basic principles.

Rehydration solutions:

- A. 40 to 50 ml/kg of rehydrating solution administered over the first 2 to 4 hours.
- B. The total volume of the rehydrating solution should not exceed 75 ml/kg/24 hours. The only exception is in cases of severe dehydration (greater than 10%) when parenteral therapy is not available. In this instance, 100 ml/kg is given over the first 4 to 6 hours.
- C. To use the rehydration solution as the maintenance solution, it must always be given in conjunction with breast milk, water, or the low sodium beverages to reduce the sodium concentration (Table 9).²⁴⁻²⁶

Maintenance solution:

After the rehydration is completed, the maintenance solution is given in the amount of 150 ml/kg/day.¹² Some of the available oral electrolyte solutions are shown in Table 10. For comparison, Table 9 provides the electrolyte and carbohydrate content of common household beverages.

In summary, the treatment of dehydration relies on the principles of a good history and physical examination, appropriate use of laboratory tests, an appropriate diagnosis and the continued reassessment of therapy.

TABLE 8.
Recommendations for Use of Oral Solutions in Treatment of Pediatric Patients With Gastrointestinal Fluid Losses*

	Rehydration Solution	Maintenance Solution
Indication for use	Treatment of acute dehydration (extracellular volume contraction)	Prevention of dehydration caused by diarrhea or hydration after treatment of dehydration
Composition		
Sodium	75 to 90 mEq/L	40 to 60 mEq/L
Potassium	20 mEq/L	20 mEq/L
Anions	20%–30% of anions as base (acetate, lactate, citrate, or bicarbonate); remainder as chloride	20%–30% of anions as base (acetate, lactate, citrate, or bicarbonate); remainder as chloride
Carbohydrate	Glucose = 2.0%–2.5% (110–140 mM/L)	Glucose = 2.0%–2.5% (110–140 mM/L)
Administration	Volume given to equal estimated fluid deficit, usually 40–50 ml/kg to be given over about 4 hours; reevaluate clinical status and therapy after 3 to 4 hours	150 ml/kg/24 hours; if additional fluid is needed to satisfy thirst, a low-solute fluid such as water or breast milk should be used

*From American Academy of Pediatrics Committee on Nutrition; use of oral fluid therapy and posttreatment feeding following enteritis in children in a developed country. *Pediatrics* 1985; 75:358–361. Reproduced by permission of *Pediatrics*.

TABLE 9.
Composition of Common Beverages*†

	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Base	CHO (gm/100 ml)
Apple juice	0.46	27.03	unk	Citrate	12.42
Awake	3.70	34.23	unk	Citrate	12.3
Club Soda	9.55	0.07	unk	Bicarbonate	0
Coca Cola	1.75	tr	26.83	Bicarbonate	10.56
Coke/diet	4.04	tr	unk	Bicarbonate	0.1
Coffee/brewed	0.48	16.90	unk	Citrate	0.3
Gatorade	22.60	2.49	17.0	Citrate	4.44
Ginger ale	3.67	0.36	unk	Bicarbonate	8.2
Grape Juice	0.91	31.41	unk	Citrate	17.5
Hawaiian Punch	9.17	6.82	unk	Citrate	12.4
Jello	25.0	0.25	10.0	Citrate	14.1
Kool-Aid	0.74	0.09	0	Citrate	10.2
Lemonade					
Canned	11.26	0.80	unk	Citrate	9.7
Frozen	4.78	10.83	unk	Citrate	10.1
Mix	0.17	1.74	unk	Citrate	9.3
Milk					
Whole 3.5%	22.43	38.03	268.28	Lactate	5.1
Lowfat 2%	22.78	38.23	268.28	Lactate	5.2
Skim	23.35	38.46	268.28	Lactate	5.3
Mountain Dew	3.78	0.64	unk	Bicarbonate	12.1
Orange juice (unsweetened)	0.48	53.90	0.83	Citrate	11.8
Pepsi Cola	1.09	0.95	unk	Bicarbonate	11.1
Pepsi/diet	7.72	0.87	unk	Bicarbonate	0.05
Seven Up	0.48	0	0	Bicarbonate	10.1
Sprite	5.74	0	unk	Bicarbonate	10.1
Tang/orange	0.26	7.23	unk	Citrate	12.5
Tea/sweetened/ice	1.61	6.80	tr	Citrate	10.3
Water/tap	2.0	0.10	0		0

*Data from references 24 to 26.

†Electrolyte composition varies with (1) the local water source used for production and/or reconstitution and (2) the various flavorings added to the product.

TABLE 10.
Oral Electrolyte Solutions: Concentration When Diluted

Product	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Base (mEq/L)	Glucose gm/L
Rehydration					
WHO oral	90	20	80	30	20
Rehydralyte (Ross)	75	20	65	30	25
Maintenance					
Infalyte (Pennwalt)	50	20	40	30	20
Lytren (Mead Johnson)	50	25	45	30	20
Pedialyte (Ross)	45	20	35	30	25
Resol (Wyeth)	50	20	50	34	34

**Special Problems: Hyponatremia, Hypernatremia,
Hypokalemia, and Hyperkalemia**

Hyponatremia

Hyponatremia will be defined as a serum sodium concentration less than 130 mEq/L. The key to understanding the pathophysiology of the hyponatremic state is the recognition that serum sodium concentration is determined by the ratio of sodium to water in the blood compartment and not by the absolute amount of either. Thus, patients with sodium deficits may develop hyponatremia, but a low serum sodium concentration can occur in patients with normal or even expanded body sodium content. In addition, although most hyponatremic patients have a decrease in the osmolality of the body fluids, hypoosmolality is not uniformly present. Some causes of hyponatremia are related to clinical situations in which the extracellular and intracellular osmolality is increased or normal, resulting in hypertonicity or isotonicity.

As in Figure 4, the differential diagnosis of hyponatremic states can be approached by logical analysis of three major pathophysiologic states that take into account changes in the tonicity of the body fluids.

Isotonic Hyponatremia

Sodium and other electrolytes are restricted to the aqueous phase of plasma, which comprises 93% of the total plasma volume. The remaining 7% consists of lipid and protein. Hyperlipidemic and hyperproteinemic states reduce sodium concentration in total plasma volume without changing its concentration in the remaining plasma water. In children, such hy-

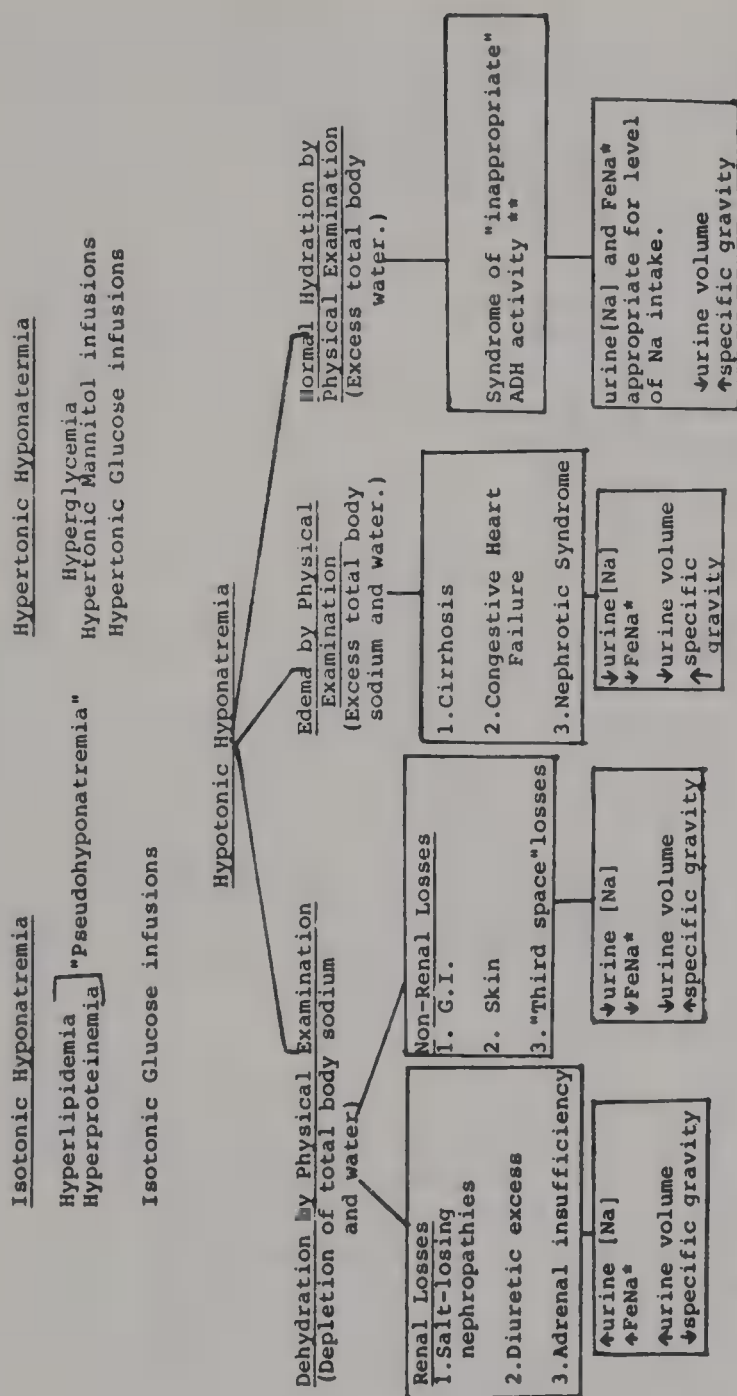


FIG 4.

Differential diagnosis of hyponatremia. *FeNa = fractional excretion of sodium. **See Table 11 for details of syndrome of inappropriate ADH activity.

perlipidemic states include the nephrotic syndrome, marginally controlled diabetes mellitus, and rare familial hyperlipidemic syndromes. The laboratory reports hyponatremia although serum osmolality remains normal. Multiplication of plasma lipid (mg/dl) by 0.002 and the increment of serum proteins above 8 gm/dl by 0.25 yields the mEq/L reduction of serum sodium caused by accumulation of these substances.²⁷

Isotonic hyponatremia can also be caused by rapid administration of large amounts of isotonic, sodium-free solutions, such as pure glucose-containing solutions (e.g., solutions of 5% dextrose). Striking hyponatremia may be present transiently, but serum osmolality remains normal.

A careful review of the patient's clinical course makes this cause of hyponatremia obvious, and the measurement of serum osmolality is not required to arrive at the diagnosis.

Hypertonic Hyponatremia

Hyperglycemia and the intravenous administration of hypertonic glucose solutions (e.g., solutions of 10% dextrose or higher) and mannitol solutions cause intracellular water to move into the extracellular space. Dilution of serum sodium and hyponatremia result, although serum osmolality is transiently elevated. As a rule, serum sodium falls by 1.6 mEq/L for each 100 mg/dl increase in blood glucose concentration.²⁸ These causes of hyponatremia become obvious upon measurement of the blood glucose concentration and review of intravenous fluids given to the patient. As in the case of isotonic hyponatremia, measurement of serum osmolality is rarely needed to identify these situations. The simplest available formula specifies that $\text{osmolality} = 2 \times [\text{Na}]$. In situations of significant hyperglycemia, the factor $[\text{glucose mg/dl}]/18$ can be added in order to account for the contribution of the elevated glucose concentration to the serum osmolality.²⁹

Hypotonic Hyponatremia

In hypotonic hyponatremia, in contrast to the previous two types of hyponatremia, sodium concentration is below normal and leads to hypoosmolality of the body fluids. In all cases, however, hyponatremia only develops in the face of continued intake (either intravenously or orally) or retention of water or other dilute solutions. As indicated in Figure 4, the differential diagnosis is based on the results of a careful physical examination with particular attention to the patient's salt and water content.

Hyponatremia in the dehydrated child is caused by diseases quite different from those related to hyponatremia in the edematous child or the apparently normally hydrated child.

The key to simplifying the differential diagnosis of hyponatremia with dehydration is the localization of sodium and water losses to renal or nonrenal sites. The history, physical examination, and selected laboratory studies clarify the source of the problem. Observation of several characteristics of the urine may also provide several helpful clues to differentiate renal from nonrenal losses.

For example, children with salt-losing nephropathies, diuretic excess, and adrenal insufficiency have a high urine output, a urine specific gravity ranging from 1.010 to 1.012, a relatively high urine sodium concentration (> 20 mEq/L), and a high fractional excretion of sodium ($> 2\%$). The fractional excretion of sodium is defined as the fraction of filtered sodium which is excreted, and is expressed as the following:

$$\frac{U_{Na}}{P_{Na}} \times GFR$$

where U_{Na} is urine sodium concentration, P_{Na} is plasma sodium concentration, GFR is glomerular filtration rate, and V is urine flow rate.

When multiplied by the factor 100, this is expressed as a percentage, and is normally $<2\%$ ($<3\%$ in the neonate). The fractional excretion of sodium is inversely related to the avidity of renal sodium conservation and since the volume terms in the equation cancel each other, it may be calculated from a random urine sample as follows³⁰:

$$\left(\frac{U_{Na}}{P_{Na}} \times \frac{P_{Cr}}{U_{Cr}} \right) \times 100$$

where U_{Na} is urine sodium concentration, P_{Na} is plasma sodium concentration, P_{Cr} is plasma creatinine concentration, and U_{Cr} is urine creatinine concentration.

Patients with dehydration due to gastrointestinal, skin, or "third space" losses have a low urine output, a high urine specific gravity (> 1.018 to 1.020), a low urine sodium concentration (10 to 20 mEq/L), and a low fractional excretion of sodium ($<1\%$).

Replacement of sodium and water deficits in all dehydrated patients with hyponatremia is based on standard principles described elsewhere in this chapter. However, the child with acute adrenal insufficiency poses a more complex problem. First, the chemical picture may be confused with acute oliguric renal failure, including azotemia, hyponatremia, hyperkalemia and acidosis. An error in differential diagnosis can lead to highly inappropriate therapy, such as fluid and salt restriction. Second, vigorous rehydration to combat hypovolemic shock must be combined with the acute administration of adrenal corticosteroids.^{31, 32} Rehydration is begun with 0.9% NaCl and 5% dextrose at 20 ml/kg administered intravenously over 30 to 60 minutes. If the patient is in shock, plasma (5 ml/kg) is required in addition to the saline solution. When acute reexpansion of the circulating blood volume has been accomplished, the rate of fluid administration continues as described earlier, but the solution used approximates the composition of 0.9% NaCl for at least the first 24 hours. Potassium is not administered until chemical and hormonal balance has been restored. Simultaneously, hydrocortisone, 2 mg/kg, is given intravenously (maximum, 100 mg) as soon as possible and as frequently as every 6 hours until the patient's condition stabilizes. At that time, the dose may be tapered over the next several days to a daily oral maintenance amount (15 to 20 mg/sq m surface area). The mineralocorticoid desoxycorticosterone is usually not required for control of the acute situation if fluid and electrolyte therapy is

appropriate. A long-acting form of this drug may be required for chronic maintenance.

Recent reports by Sterns et al.³³ and Arieff³⁴ draw attention to the possibility that overly rapid correction of hyponatremia may lead to central pontine myelinolysis. However, the incidence of this devastating neurologic complication in children is extremely low, and its cause and effect relationship to the treatment of hyponatremia is controversial.³⁵ There are no data on which to base a firm recommendation for a safe rate of correction, although various sources suggest rates such as 5 mEq/kg/hour or 2 mEq/L/hour.³⁵

The pathophysiology of hyponatremia in children with edema involves the excessive reabsorption of sodium and water, with disproportionately high water retention. Cirrhosis of the liver, congestive heart failure, and nephrotic syndrome are three clinical entities producing this situation. They are thought to have in common a decreased effective circulating blood volume that results in increased renal salt and water retention. These conditions are obvious if a careful history, physical examination, and appropriate laboratory tests are obtained.

A careful analysis of the urine is useful in confirming that the expected physiologic mechanisms are operative. Children with hyponatremia due to these conditions have a low urine output, a high urine specific gravity, a low urine sodium, and a low fractional excretion of sodium, all very similar to the urinary findings in the child with dehydration due to nonrenal losses. The initial difference between the two groups is the presence of physical signs of volume contraction in one, and edema in the other.

Appropriate treatment for this group of children includes therapy of the basic disease and restriction of salt intake. Water intake may also need to be restricted in some, and diuretics may be required. Nephrotic children with massive edema and hyponatremia often respond to salt restriction with either diuresis alone or in combination with the intravenous infusion of hypertonic albumin (1 gm/kg given over 60 minutes, up to three times in a 24-hour period).³⁶

Some hyponatremic patients show no evidence of volume depletion or expansion on physical examination. In these cases, hyponatremia is due to expansion of total body water, and this water overload is not obvious to examination because the majority of the excess is located in the intracellular space. As shown in Figure 4, the major cause of this in children is the syndrome of inappropriate antidiuretic hormone (ADH) activity. In this syndrome, positive water balance is caused by water retention secondary to ADH secretion stimulated by factors other than hypovolemia or hyperosmolality. Many nonosmotic stimuli to ADH release have been described in children, and these risk factors are listed in Table 11, which also includes a list of drugs causing the same syndrome by augmenting the renal action of ADH.^{27, 28} In addition to these causes, the same syndrome has been reported in premature infants with late hyponatremia,³⁷ newborns with water intoxication due to nebulization with nasal CPAP (continuous positive

TABLE 11.
Causes of the Syndrome of Inappropriate
Antidiuretic Hormone Activity

Stress, anxiety
Trauma (including postoperative states)
Pulmonary: Infections, asthma, positive pressure ventilation, tumors
Central nervous system: Infections, tumors, hemorrhage, stroke
Cardiac: Arrhythmias, surgery
Neoplasms: Pulmonary, central nervous system, gastrointestinal, bone, genitourinary, lymphoma
Metabolic: Hypothyroidism, hypoadrenalism
Drugs
Stimulate release of ADH
Cyclophosphamide
Vincristine
Barbiturates
Opiates
Clofibrate
Nicotine
Histamine
Carbamazepine
Diuretics
Augment renal action of ADH
Chlorpropamide
Tolbutamide
Phenformin

airway pressure),³⁸ and even apparently normal infants receiving feedings of dilute formulas.³⁹

Suspicion of this syndrome can be strengthened in the face of a low urine volume with a relatively high urine specific gravity. Although classically the urine is more concentrated than the blood when ADH expression is at its fullest, many intermediate situations occur in which the major clue is finding a urine that is not maximally dilute (i.e., the urine is "relatively" concentrated). Since maximal dilution implies a urine specific gravity < 1.003 (consistent with a urine osmolality < 75 mOsm/L), the physician should not hesitate to suspect this syndrome in the appropriate circumstances even in the face of a relatively dilute urine. For example, a urine osmolality of 150 mOsm/L (consistent with a urine specific gravity ~ 1.005) in association with a plasma osmolality of 240 mOsm/L (seen in a patient with a serum sodium concentration of 120 mEq/L) is compatible with the syndrome of inappropriate ADH activity. Sodium handling is not impaired in these patients, and sodium excretion is appropriate for the level of sodium intake. As a result, urinary sodium concentration and the fractional excretion of sodium can range widely within the "normal" range.

The mainstay of therapy includes discontinuation of the offending drug, treatment of the underlying disease, amelioration of the stressful event,

and generation of a negative water balance. This is usually produced by strict reduction of water intake to levels equal to the daily insensible losses or less (i.e., <400 to 500 ml/sq m). In emergency situations in which seizures or other severe neurologic complications have developed, or serum sodium concentration is so low as to threaten seizures (<120 mEq/L), treatment includes intravenous administration of sufficient hypertonic NaCl (3% or 5%) to raise the serum sodium concentration to 125 to 130 mEq/L. The dose needed to correct to 130 meq/L is calculated as follows:

$$(130 - A) \times 0.6 \times B.W$$

where:

A = observed serum sodium concentration

BW = body weight

Since 3% sodium chloride contains sodium in a concentration of approximately 500 mEq/L or 0.5 mEq/ml, the volume required in ml is equal to twice the number of mEq desired. Half the number of mEq calculated are given over 1 to 2 hours, followed by the remainder of the dose if required. The administration of a combination of intravenous furosemide and hypertonic saline is occasionally useful in patients resistant to more conservative therapy or with impending congestive heart failure. In these cases, hypertonic saline is given in a dose calculated to raise the serum sodium concentration as previously described, and additional doses are given hourly to match the amount of sodium excreted by the induced diuresis.⁴⁰

Hypernatremia

Hypernatremia will be defined as a serum sodium concentration greater than 150 mEq/L. Hypernatremic disorders are divided into two categories based on the physical examination and an estimate of the body fluid status (Fig 5). Differential diagnosis of any of the abnormalities of serum sodium concentration depends primarily on the results of a complete physical examination.

Hypernatremia in children commonly is associated with conditions leading to loss of body sodium and water or isolated water loss. In any case, water loss is greatly out of proportion to sodium loss. Weight loss is evident, and physical signs of volume contraction are present if significant depletion of sodium has occurred. As seen in Figure 5, this type of hypernatremia can be caused by renal or extrarenal losses of sodium and water. In all of these situations, the problem is exacerbated if replacement solutions are relatively high in sodium content. The common pathophysiologic factor in most chronic renal diseases, including obstructive uropathy, is a concentrating defect and a continued inappropriate excretion of water and sodium, in spite of dehydration. This defect results in a continued generous urine output (even in the presence of obvious volume contraction), with low urine specific gravity (< 1.015) and relatively high urine sodium concentration (> 20 mEq/L).

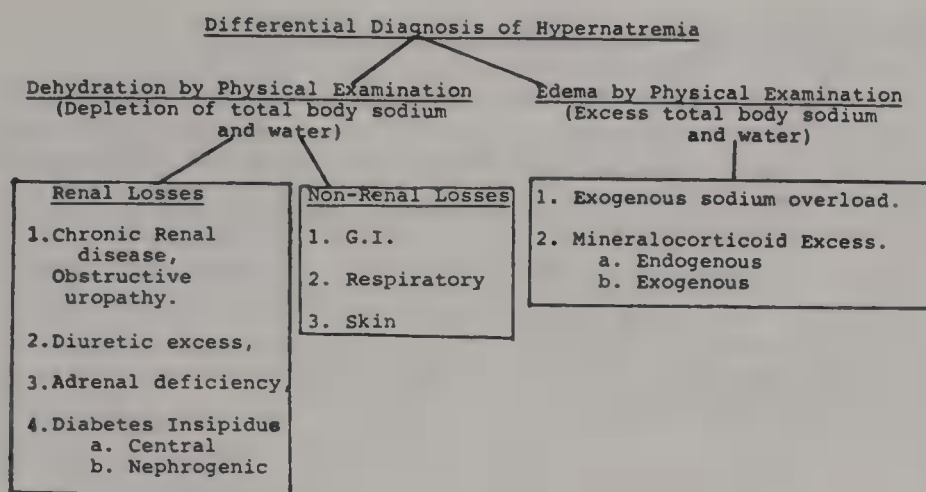


FIG 5.

Differential diagnosis of hypernatremia.

Central diabetes insipidus and hereditary nephrogenic diabetes insipidus, are marked by almost pure water loss, with maintenance of normal sodium balance. Peripheral signs of dehydration are less, for the same degree of body weight loss, than in the previously described conditions, because there is no sodium depletion. The urine output is large, with a low urine specific gravity (<1.005). These two conditions can be differentiated based on the response to a standard dose of ADH given after a period of water deprivation.^{41, 42} In small children, the risk of developing symptomatic volume depletion during the thirsting period mandates that this test be performed under close medical supervision, with intravenous rehydrating solutions close at hand and avoidance of an acute body weight loss of more than 3% to 5%.

Extrarenal causes of hypernatremia include vomiting, diarrhea, tachypnea (due to pulmonary infections, compensation for metabolic acidosis, etc.), and excessive skin losses (due to fever, increased insensible losses in the premature infant, etc.). Respiratory losses usually are not accompanied by sodium, but skin losses may include more or less sodium, depending, for example, on whether the child has cystic fibrosis. The urine output is low in these patients, with a high urinary specific gravity (>1.018 to 1.020) and a low urine sodium concentration (<20 mEq/L). In this respect, the renal response is as expected in any case of dehydration due to extrarenal losses. The premature infant may not be able to produce a concentrated urine under these circumstances.

Treatment of hypernatremia due to sodium and water losses, with resultant volume contraction, is a matter of applying the standard principles of rehydration described earlier. Diuretics are discontinued if they are a major cause of the problem, and adrenal deficiency requires specific hormonal replacement in addition to volume expansion. The major compli-

cations of the treatment of hypernatremia are related to the central nervous system.^{43, 44} The dehydration itself can cause subarachnoid and subdural hemorrhage, sagittal sinus thrombosis, and seizures. Excessively vigorous rehydration may result in cerebral edema, manifest by increased intracranial pressure, seizures, and stupor. In general, the proper volume and rate of administration of the rehydrating solution are of greatest concern, while the exact composition of the solution is less important.⁴³

Sodium overload is a less common cause of hypernatremia in children. The history of an excessive intake of salt is usually obvious, and the physical examination may show signs of volume expansion such as edema and hypertension. Iatrogenic causes of this type of hypernatremia include par-enteral administration of large doses of a hypertonic sodium bicarbonate solution, or hypertonic sodium chloride (3% or 5%) given inadvertently in place of a 5% Dextrose solution. Also, errors in mixing infant feeding formulas from powdered salt and sugar supplements can lead to severe hypernatremia.

Appropriate treatment must take place rapidly, and includes administration of 5% dextrose in water simultaneously with the intravenous administration of a potent natriuretic agent such as furosemide.⁴⁵ The infusion rate varies depending on the rate of diuresis, the rate of decrease in serum sodium concentration, and the neurologic status. Excessive water replacement can produce cerebral edema. Patients with congestive heart failure or oliguria who do not respond to these measures may require dialysis.

Children with one of the conditions caused by an excess of mineralocorticoids often prove to be diagnostic challenges but frequently have only mild hypernatremia in the upper normal range. Appropriate treatment of the primary disease or a decrease in the sodium intake and a modification of the dose of mineralocorticoid being administered can be expected to lead to improvement.

Hypokalemia

Hypokalemia will be defined as a serum potassium concentration less than 3.5 mEq/L. Concentrations below this level imply either decreased body potassium stores due to decreased intake or external losses, or redistribution of extracellular potassium into the intracellular space (Table 12). External losses take place via the gastrointestinal, skin, or renal routes. Increased intracellular uptake is stimulated by alkalosis, hyperinsulinism (as seen in malnourished patients during initiation of total parenteral nutrition), the "pseudohypokalemia" of leukemia (caused by avid white blood cell uptake of potassium), and the rare entity hypokalemic periodic paralysis.⁴⁶

Once appropriate evaluation has ruled out decreased intake or redistribution into cells as the cause of hypokalemia, the history, physical examination, and selected laboratory tests are used to determine the site of potassium loss from the body. The gastrointestinal losses listed in Table 12 should be obvious from history and physical examination. Urinary potassium concentration is low (<20 mEq/L) in most of these situations, but

TABLE 12.
Differential Diagnosis of Hypokalemia

Decreased Potassium Stores	Normal Potassium Stores
Decreased intake	Redistribution
High carbohydrate diet	Alkalosis
Anorexia nervosa	Hyperinsulinism
	Leukemia ("pseudohypokalemia")
	Hypokalemic periodic paralysis
External Losses	
Gastrointestinal	
Malabsorption	
Vomiting	
Gastric suction	
Laxative/enema abuse	
Diarrhea	
Skin	
Renal	
Hypertensive	
Renovascular disease	
Renin-producing tumors	
Adrenal adenomas	
Congenital adrenal hyperplasia	
Cushing syndrome	
Exogenous mineralocorticoids	
Normotensive	
Renal tubular acidosis	
Fanconi's syndrome	
Chronic interstitial and cystic	
renal disease	
Bartter's syndrome	
Liddle's syndrome	
Antibiotics	
Diuretics	

may be higher in the face of vomiting and the kaliuresis of metabolic alkalosis. Skin losses of potassium are usually minor, but may become significant in children exercising heavily in hot weather. Urinary potassium concentration is also low in this situation.

A problem in the differential diagnosis of hypokalemia involves the long list of causes of renal potassium loss. The diagnostic process may be simplified by separating the various conditions into those associated with hypertension (due to hyperreninemia and/or hyperaldosteronism) and those with normal blood pressure. The former include the causes of renovascular

hypertension, renin-producing tumors, adrenal adenomas and hyperplasia, some forms of congenital adrenal hyperplasia, Cushing's syndrome, and exogenous mineralocorticoid administration. The latter involve conditions such as proximal and distal renal tubular acidosis, diseases resulting in Fanconi's syndrome of multiple proximal tubular dysfunction, rare cases of chronic interstitial and cystic renal disease, Bartter's syndrome, Liddle's syndrome, administration of diuretics, intake of tubulotoxic antibiotics (gentamycin, amphotericin), or antibiotics which act as relatively nonreabsorbable anions (carbenicillin). In all of these cases, urinary potassium concentration is high (>20 mEq/L).

The symptoms and signs of hypokalemia in general do not appear until the serum concentration drops below 3 mEq/L. Persistent hypokalemia can lead to the following complications: (1) metabolic abnormalities such as hyperglycemia and carbohydrate intolerance, (2) renal concentrating defects leading to polyuria and polydipsia, as well as sodium retention and edema, and (3) a host of neuromuscular disorders including generalized weakness, paralysis, lethargy, confusion, intestinal ileus, autonomic insufficiency with orthostatic hypotension, and cardiac arrhythmias.⁴⁶⁻⁴⁸

Because the intracellular stores of potassium cannot be easily measured and there is no close relationship between the serum concentration and total body potassium content, replenishment of potassium deficits is performed empirically without specific calculations. It is important to avoid sudden large intravenous potassium loads and to replace deficits slowly over several days. If repair of deficits must be initiated quickly because of life-threatening complications, urine output should be assured and electrocardiographic monitoring must be available.

Oral replacement of deficits is safest, and a reasonable dose is 3 mEq/kg/day in addition to maintenance requirements.⁴⁶ The solutions available for oral use include the chloride, bicarbonate, phosphate, gluconate, citrate, and acetate salts of potassium. These liquids are more easily tolerated by the gastrointestinal tract than the solid forms of potassium supplements. Although the chloride salt may be more effective, the organic anion salts are more pleasant tasting and better accepted by young children. If intravenous potassium replacement must be used, the concentration should be limited to a maximum of 40 mEq/L unless serious, life-threatening complications of hypokalemia are present. In such cases, even higher concentrations can be used, but only with careful monitoring of the heart rate and rhythm.⁴⁶⁻⁴⁸

Hyperkalemia will be defined as a serum potassium concentration greater than 5.5 to 6.0 mEq/L (Table 13). In contrast to the hypokalemic syndromes, in which factitious causes are rare, "pseudohyperkalemia" is relatively common and must be ruled out before embarking on intensive investigation and treatment of hyperkalemia. For example, leucocytosis above 100,000/cu mm and a platelet count greater than 750,000/cu mm can result in elevation of serum potassium concentration via release of intracellular potassium during clotting in the test tube.^{28, 49} A measurement

TABLE 13.
Differential Diagnosis of Hyperkalemia

Elevated Potassium Stores	Normal Potassium Stores
Increased Intake	Pseudohyperkalemia
Endogenous	Leucocytosis
Hemolysis	Thrombocytosis
Rhabdomyolysis	Test tube hemolysis
Exogenous	Ischemic blood drawing
Aged blood	Redistribution
Salt substitutes	Metabolic acidosis
K+ Penicillin	Insulin deficiency
Decreased Excretion	Hyperkalemic periodic paralysis
Acute or chronic renal failure	
Deficiency of renin-angiotensin-aldosterone system	
Adrenal insufficiency	
Adrenal 21-OH'lase deficiency	
Hyporeninemic hypoaldosteronism	
Pseudohypoaldosteronism	
Drug inhibitors: captopril, β -blockers	
Renal tubular K+ secretory defects	
Sickle cell nephropathy	
Systemic lupus	
Congenital (Spitzer) syndrome of short stature and acidosis	
Potassium-sparing diuretics	
Spironolactone	
Triamterene	
Amiloride	

of plasma potassium collected in a heparinized tube eliminates this problem. Similarly, ischemic blood drawing with a tight tourniquet and test tube hemolysis of red blood cells may lead to factitious hyperkalemia. In each of these cases, an electrocardiogram shows no evidence of hyperkalemic changes.

True hyperkalemia is associated with (1) redistribution of intracellular potassium to the extracellular space, (2) decreased excretion of potassium as a consequence of the diseases listed in Table 13, and (3) an acute increase in potassium intake in the face of an excretory defect.

Among the causes of redistribution hyperkalemia, the most common in children is metabolic acidosis. In contrast to respiratory acidosis, which has little effect, metabolic acidosis results in a rise of at least 0.5 to 1.5 mEq/L in the potassium concentration, depending on the severity of the acid-base disturbance.⁴⁹

In the majority of situations in pediatrics, true hyperkalemia is caused by

impaired potassium excretion because of severe acute or chronic renal failure with oligo-anuria. Less commonly, defects in the renin-angiotensin aldosterone system, as listed in Table 13, are involved. As more hypertensive children are treated with β -blockers or angiotensin-converting enzyme inhibitors such as captopril, the incidence of hyperkalemia may increase. Particularly interesting is the recognition of hyporeninemic hypoaldosteronism in children. This condition is well described in the aging diabetic population,^{50, 51} but is becoming more frequently diagnosed in younger patients with severe renal interstitial disease of almost any cause.⁴⁹ The renal diseases that may present with specific tubular potassium secretory defects are uncommon but not rare in children, and their likelihood of causing hyperkalemia is especially great when they progress to renal functional impairment.

Although the endogenous and exogenous sources of dangerously high potassium loads must be kept in mind, they do not generally cause acute hyperkalemia in the absence of excretory defects. For example, only children with renal failure, adrenal insufficiency, or spironolactone therapy are at particular risk of life-threatening hyperkalemia when treated with aged blood transfusions or a large dose of potassium penicillin. Similarly, only if massive rhabdomyolysis results in acute oligoanuric renal failure is there serious risk of acute hyperkalemia due to the release of muscle potassium stores.

The risks of acute hyperkalemia include muscle weakness and paralysis, usually beginning in the legs, as well as cardiac ventricular arrhythmias and cardiac arrest. Occasionally, cardiac involvement develops before skeletal muscle weakness, and death may occur unexpectedly.^{47, 48} The electrocardiographic abnormalities of hyperkalemia are initially a tall peaked T wave in the precordial leads, followed by decreased amplitude of the R wave, widened QRS complex, prolonged PR interval, disappearance of the P wave, and finally a blending of the QRS complex into the T wave, forming the classic sine wave of hyperkalemia.⁵²

Acute hyperkalemia is a medical emergency that must be treated immediately. The therapy described here should be initiated whenever skeletal muscle or cardiac abnormalities are present, no matter what the degree of hyperkalemia. Otherwise, particularly at concentrations of potassium between 6 and 7 mEq/L, the first phase of the treatment protocol may not be required, and limitation of potassium intake and slow removal by exchange resin, as described here, will usually suffice.

The first phase of management is the emergency restoration of a normalized ratio of intracellular to extracellular potassium concentration. When this is successful, the skeletal muscle and cardiac function temporarily revert to normal. Therapy involves administration of (1) sodium bicarbonate, 2 mEq/kg body weight intravenously as a rapid push over 3 to 5 minutes, (2) 10% calcium gluconate, 0.5 to 1.0 ml/kg body weight intravenously as a rapid push over 3–5 minutes, and (3) 50% glucose, 1 ml/kg body weight intravenously over 30 minutes accompanied, in some cen-

ters, by regular insulin, 1 unit for every 3 gm of glucose. The endogenous insulin response to the hypertonic glucose may be sufficient to obviate the need for exogenous insulin. The effectiveness of bicarbonate and glucose/insulin therapy lasts for several hours, whereas that of the calcium infusion has a duration of only 30 minutes. In addition, transient electrocardiographic improvement can be seen almost immediately after administration of the sodium bicarbonate.

The second phase of treatment removes excess potassium from the body in a slow, controlled fashion. Sodium polystyrene sulfonate resin (Kayexalate), a sodium/potassium cation exchanger, is administered orally or as a retention enema over 30 to 45 minutes at a dose of 0.5 to 1.0 gm/kg body weight. To prevent constipation and to induce further stool losses of water and potassium, the resin is administered in a 20% to 25% sorbitol solution, 3 ml of solution for each 1 gm of resin. This combination should be used with care because the osmotic cathartic effect of sorbitol in higher concentrations (70%) has been associated with severe hypernatremic dehydration.⁵³ The Kayexalate may be repeated every 4 to 6 hours as needed. Frequent use, however, results in progressive sodium overload because of the ion exchange process.

Finally, if these measures are not successful, or are not effective quickly enough because of accompanying renal failure, acute peritoneal or, preferably, hemodialysis must be instituted.^{47, 48}

References

1. Winters RW (ed): *Principles of Pediatric Fluid Therapy*, ed 2. Boston, Little, Brown & Co, 1982.
2. Darrow DC: *A Guide to Learning Fluid Therapy*. Springfield, IL, Charles C Thomas, Publisher, 1964.
3. Holliday MA, Segar WE: Maintenance need for water in parenteral fluid therapy. *Pediatrics* 1957; 19:823.
4. Holliday MA: Fluid and nutrition therapy, in Holliday MA, Barratt TM, Vernier RL (eds): *Pediatric Nephrology*, ed 2. Baltimore, Williams & Wilkins Co, 1987, pp 173–180.
5. Yared A, Ichikawa I: Renal blood flow and glomerular filtration rate, in Holliday MA, Barratt TM, Vernier RL (eds): *Pediatric Nephrology*, ed 2. Baltimore, Williams & Wilkins Co, 1987, pp 45–58.
6. Darrow DC: The significance of body size. *Am J Dis Child* 1959; 98:416.
7. Finberg L, Kravath RE, Fleischman AR (eds): *Water and Electrolytes in Pediatrics, Physiology, Pathophysiology and Treatment*. Philadelphia, WB Saunders Co, 1982.
8. Friis-Hansen BJ: Body water compartments in children. *Pediatrics* 1961; 28:171.
9. Weil WB Jr, Bailie MD (eds): *Fluid and Electrolyte Metabolism in Infants and Children: A Unified Approach*. New York, Grune & Stratton, Inc, 1977.
10. Dubois EF: The basal metabolism in fever. *JAMA* 1921; 77:352.
11. Fomon SJ (ed): *Infant Nutrition*, ed 2. Philadelphia, WB Saunders Co, 1974.

12. Lattanzi WE, Siegel NJ: A practical guide to fluid and electrolyte therapy. *Curr Probl Pediatr* 1986; 16(1):1-43.
13. Goldberger E: *A Primer of Water, Electrolyte and Acid-Base Syndromes*. Philadelphia, Lea & Febiger, 1986.
14. Darrow DC, Pratt EL: Fluid therapy; Relation to tissue composition and the expenditure of water and electrolyte. *JAMA* 1950; 143:432-439.
15. Wood RE, Boat TF, Doershuk CF: Cystic fibrosis, in Murray JD (ed): *Lung Disease, State of the Art*. New York, American Lung Association, 1975-1976, p 275.
16. Goodman LS, Gilman A: *The Pharmacological Basis of Therapeutics*, ed 4. New York, Macmillan Publishing Co, 1970, p 852.
17. Segar WE: Parenteral fluid therapy. *Curr Probl Pediatr* 1972; 3:3-40.
18. Nash MA: *Water and Solute Homeostasis in Pediatrics*, Kidney Disease, Edelmann CM Jr (ed), Boston, Little, Brown & Co, 1978, pp 290-306.
19. Valtin H: *Renal Dysfunction: Mechanisms Involved in Fluid and Solute Balance*. Boston, Little, Brown & Co, 1979, p 18.
20. Finberg L: Treatment of dehydration in infancy. *Peds Rev* 1981; 3:113-120.
21. Thurston JH, Hauhart RE, Dirges JA: Taurine: A role in osmotic regulation of mammalian brain and possible clinical significance. *Life Sci* 1980; 26:1561.
22. American Academy of Pediatrics Committee on Nutrition: Use of oral fluid therapy and posttreatment feeding following enteritis in children in a developed country. *Pediatrics* 1985; 75:358-361.
23. Oral fluids for dehydration. *Medical Letter* 1987; 29:63.
24. Adams CF: Nutritive value of American foods, in *USDA Handbook*, no. 8 and no. 456. Washington, DC, 1975.
25. Leveille GA, Zabik ME, Morgan KJ: *Nutrients in Foods*. Cambridge, MA, The Nutrition Guild, 1983.
26. Geigy scientific tables, units of measurement, body fluids, nutrition, in *Health Sciences*. West Caldwell, NJ, Ciba Geigy, 1981, vol 1.
27. Weitzman R, Kleeman CR: Water metabolism and the neuro-hypophyseal hormones, in Maxwell MH, Kleeman CR (eds): *Clinical Disorders of Fluid and Electrolyte Metabolism*. New York, McGraw-Hill Book Co, 1980, pp 531-646.
28. Narins RG, Jones ER, Stom MC, et al: Diagnostic strategies in disorders of fluid, electrolyte, and acid-base homeostasis. *Am J Med* 1982; 72:496-520.
29. Fanestil DD: Hypoosmolar syndromes, in Andreoli TE, Grantham JJ, Rector FC Jr (eds): *Disturbances in Body Fluid Osmolality*. Bethesda, Md, American Physiological Society, 1977, pp 267-284.
30. Espinel CH: The FeNa test: Use in the differential diagnosis of acute renal failure. *JAMA* 1976; 236:579-582.
31. Migeon CJ, Lanes R: Adrenal cortex: Hyper- and hypofunction, in Lifshitz F (ed): *Pediatric Endocrinology, A Clinical Guide*. New York, Marcel Dekker, 1985, pp 189-190.
32. Bongiovanni AM: The Adrenal cortex, in Kaplan SA (ed): *Clinical Pediatric and Adolescent Endocrinology*. Philadelphia, WB Saunders Co, 1982, p 179.
33. Sterns RH, Riggs JE, Schochet SS Jr: Osmotic demyelination syndrome following correction of hyponatremia. *N Engl J Med* 1986; 214:1535-1542.
34. Arieff AI: Hyponatremia, convulsions, respiratory arrest, and permanent brain damage after elective surgery in healthy women. *N Engl J Med* 1986; 314:1529-1535.
35. Narins RG: Therapy of hyponatremia: Does haste make waste? *N Engl J Med* 1986; 314:1573-1575.

36. Weiss RA, Schoeneman MJ, Greifer I: Treatment of severe nephrotic edema with albumin and furosemide. *NY J Med* 1984; 84:384–386.
37. Sulyok E, Kovacs L, Lichardus B, et al: Late hyponatremia in premature infants: Role of aldosterone and arginine vasopressin. *J Pediatr* 1985; 106:990–994.
38. Rosenfeld WN, Linshaw M, Fox HA: Water intoxication: A complication of nebulization with nasal CPAP. *J Pediatr* 1976; 89:113–114.
39. David R, Ellis D, Gartner JC: Water intoxication in normal infants: Role of antidiuretic hormone in pathogenesis. *Pediatrics* 1981; 68:349–353.
40. Hantman D, Rossier B, Zohlman R: Rapid correction of hyponatremia in the syndrome of inappropriate secretion of antidiuretic hormone. *Ann Int Med* 1973; 78:870–875.
41. Robertson GL, Shelton RL, Athar S: The osmoregulation of vasopressin. *Kidney Int* 1976; 10:25–37.
42. Miller M, Kalkos T, Moses AM, et al: Recognition of partial defects in antidiuretic hormone secretion. *Ann Int Med* 1970; 73:721–729.
43. Hogan GR: Hyponatremia: Problems in management. *Pediatr Clin N Am* 1976, vol 23, pp 569–574.
44. Finberg L: Pathogenesis of lesions in the nervous system in hypernatremic states: I. Clinical observations of infants. *Pediatrics* 1959; 25:40–45.
45. Feig PU, McCurdy DK: The hypertonic state. *N Engl J Med* 1977; 297:1444–1454.
46. Linshaw MA: Potassium homeostasis and hypokalemia. *Pediatr Clin N Am* 1987; 34:649–681.
47. Schultze RG, Nissenson AR: Potassium-physiology and patho-physiology, in Maxwell MH, Kleeman CR (eds): *Clinical Disorder of Fluid and Electrolyte Metabolism*. New York, McGraw-Hill Book Co, 1980, pp 113–145.
48. Gabow P: Disorders of potassium metabolism, in Schrier RW (ed): *Renal and Electrolyte Disorders*. Boston, Little, Brown & Co, 1976, pp 143–167.
49. DeFronzo RA, Bia M, Smith D: Clinical disorders of hyperkalemia. *An Rev Med* 1983; 33:521–554.
50. DeFronzo RA: Hyperkalemia and hyporeninemic hypoaldosteronism. *Kidney Int* 1980; 17:118–134.
51. DeChatel R, Weidmann P, Flammer J, et al: Sodium, renin, aldosterone, catecholamines, and blood pressure in diabetes mellitus. *Kidney Int* 1977; 12:412–421.
52. Fisch C: Relation of electrolyte disturbances to cardiac arrhythmias. *Circulation* 1973; 47:408–410.
53. Farley GD: Severe hypernatremic dehydration after use of an activated charcoal: Sorbitol suspension. *J Pediatr* 1986; 109:719–722.

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